

Seeking a fortune in space

The US Administration would have corporations look to Earth orbit for new technology. It will have succeeded only when companies are spending their own funds, not NASA's subsidies.

HAD things gone as planned, the space shuttle Discovery would have spent last week in orbit with a most unusual passenger on board. Mr Charles Walker, not an astronaut, but an employee of the McDonnell Douglas Corporation, was to conduct a major test of the electrophoresis equipment which, his company hopes, will exploit the weightlessness of space to manufacture a very pure hormone for the treatment of diabetes. Although the precise nature of the experiment and the product remains a company secret, it is viewed in industrial circles as the symbolic harbinger of a lucrative space-based manufacturing industry which some expect to earn \$8,000 million a year by the middle of the next decade. The technical failures that forced the National Aeronautics and Space Administration (NASA) to postpone Discovery's maiden flight therefore provide a salutary reminder of the difficulties that still lie ahead.

Why salutary? Because the prospect of exploiting the special properties of space to make money has suddenly become intricately linked to the destiny of NASA, and therefore to the future of the space programme itself. The commercialization of space is, after all, the principal element in the still fuzzy rationale for President Reagan's decision this year to bless NASA's third great adventure in space, the construction of a permanently-manned space station by 1992. To be sure, there are other elements, such as the hope that the space station might become a way-station for man's return to the Moon or for voyages to distant planets. There is also an argument — promoted by NASA but belittled by the Pentagon — that a space station will enhance the security of the United States. But the argument that finally won the day for NASA was its contention that a space station would become, early in the next century, the cornerstone of a visionary new manufacturing sector in which the United States' lead could be made unassailable.

Investment

NASA's triumph over the Pentagon, the Office of Management and Budget and the space science community, all of which opposed the space station, may well prove historic. It has long been an axiom within the agency that without a major engineering challenge, such as the Apollo programme or the development of the shuttle, NASA would be hard pressed to maintain its extraordinary capacity for innovation. And inasmuch as space science and exploration depend on brilliant engineering, what is good for NASA is indeed good for the United States. But the successful political struggle for permission to build the space station has left NASA with an awkward legacy — a vested interest in persuading the administration and Congress, as well as the taxpayers of the United States, that investments in space can be made to pay for themselves.

In the case of some kinds of investment, the proposition is self-evident. The private sector has already successfully exploited what might be called the geometric properties of space, the ability of satellites at high altitude to link the Earth with line-of-sight messages. There are already so many civilian communications satellites in orbit, generating more than \$1,000 million a year in revenues, that some orbital slots in segments of the Equator are becoming overcrowded. Nor is there any sign yet of an end to the discovery of new commercial applications for such satellites. To applications such as direct broadcasting will soon be added the

development of cheap satellite telephones for countries without the hard-wiring infrastructure necessary for conventional telephones. And the use of satellite-based navigation systems such as Geostar may soon be combined with the technology of the video-disc to provide motorists and sailors with a map displaying their position to the nearest metre.

The success of private communications satellites has spawned other profitable ventures in space, in the form of a thriving industry for support services. They range from the data collection and analysis services which small specialized corporations offer consumers of Earth-observation photographs to the growing interest of aerospace companies in building their own launch vehicles and spacecraft to compete with the shuttle. In this case, however, the pace of commercialization has been moderated by high development costs, and it is doubtful whether the private sector can flourish without initial public subsidies, however indirect. Thus NASA is already being nagged by companies which want to launch satellites on the US Air Force's cast-off expendable rockets to raise its price for shuttle rides. And the seven companies that have asked to take over the United States' Landsat remote-sensing satellites will do so only if they are subsidized for the several years it will take them to nurture a worthwhile market.

Space factories

Making things in space and bringing them back to Earth is another matter altogether. A factory 200 miles above the Earth would, it is true, enjoy the advantages of unfiltered solar radiation, a near-perfect vacuum and the almost total absence of gravity. The effects of convection, sedimentation and hydrostatic pressure, which complicate terrestrial manufacture, could be avoided. The containerless manipulation of molten substances, held together by their own surface tension, holds out the promise of eliminating impurities and geometric imperfections. The space-based products now being explored by many companies range from pure pharmaceuticals to space-grown mercury cadmium telluride crystals that can be used in sensor arrays for military applications.

But disadvantages abound. The cost of getting equipment into space and returning products to Earth makes space the last place one would choose to manufacture anything if an alternative was available. Although these costs can be expected to fall as launch technologies mature and competition between the shuttle and Ariane sharpens, last week's failure of Discovery suggests the process will be gradual. Meanwhile, whatever is manufactured in space must be sold at an alarmingly high price — between \$100,000 and \$1 million per kilogram, according to a recent study — if it is to pay for itself. That imposes severe limits on the kinds of products worth exploring. A miracle cure for cancer or diabetes would find buyers at any price, but the demand for most industrial components, even in electronics or speciality materials, is exquisitely price-elastic, particularly if large volumes are required.

There is a second constraint. Until the space station is built, or some of the commercially developed free-flying platforms become available, industrial endeavours must depend on the shuttle. That means that companies must make do with a maximum of ten days in orbit and only five kilowatts of power.



They have also to put up with periodic bumps as the shuttle is manoeuvred, whereas most space-manufacturing processes, such as growing crystals, call for long periods in a stable environment. That so many companies have nevertheless been prepared to experiment may testify less to their faith in the future of space manufacturing than to the alluring incentives — ranging from free shuttle rides to access to NASA laboratories — that they are being offered.

If NASA has its way, these incentives will grow. A report on space commercialization being mulled over in the White House says that the hoped-for private sector investment in space will not materialize without a massive subsidy in the form of tax credits and risk-sharing agreements. Both NASA and the aerospace industry, however, have a strong vested interest in making the future of space manufacturing look as rosy as possible. Whether there are any real grounds to expect a fair return from a massive public investment is another question. □

Weapons in space

The Soviet and US Governments should return to private diplomacy over space weapons.

WHEN the future of the world may seem to hang on the conversation at a White House barbecue between Mr George Shultz, the US Secretary of State, and Mr Anatoli Dobrynin, the Soviet ambassador to Washington, the time has surely come when ordinary mortals may wonder whether it is not high time that governments conducted their affairs professionally. Hopefully, people have been surmising that the two men were trying to make sense of the latest misunderstanding between the Soviet Union and the United States on arms control. The Soviet Government had issued an invitation to a conference in Vienna in September to negotiate a ban on weapons in space, the United States had complicated its swift acceptance with the statement that it would wish to talk about nuclear weapons as well, the Soviet Union had accused its opposite number of not being "serious" and the possibility that talks might take place was once more up in the air. Whether Shultz and Dobrynin were trying to sort out the muddle or merely planning a fishing trip, time will no doubt tell.

The charge that both governments are behaving unprofessionally is true in the particular sense that they have fallen back on the use of public statements to the international press for doing what ambassadors and other diplomats are employed for. For the past several years, negotiation by press release has been a constant source of trouble. The Geneva talks on nuclear weapons of intermediate range, which began with both sides taking trappist vows, were quickly undermined by the publication of "negotiating positions" which, once public, ceased to be negotiable. That illustrates the most persuasive reason why diplomatic negotiations should, to begin with, be private. Another is that negotiation by public statement entails attempts to capture public support which, if successful, anger the other side. This is nicely illustrated by last weekend's fracas.

The substance of the argument between the Soviet Union and the United States on space weapons is likely to be hopelessly obscured by these events. Two kinds of weapons are involved — devices for destroying predictable Earth satellites (now almost realities) and more futuristic star wars weapons, meant for destroying warheads. The case for banning both kinds of systems is straightforward — their deployment by one side would give the other the sense that the strategic balances had become disadvantageously inequitable. But could such an agreement be verified? That is what the US Administration has been asking, properly enough. The simple answer is that, within reasonable limits, it is. The simplest way of arranging this is to require that each side should tell the other the purpose of all rockets fired in such a way as to carry objects into orbits about the Earth or above an altitude of, say, 100 km. That sometimes they would find themselves acknowledging that many Earth satellites have a military function would be no surprise, and an uncovenanted benefit of agreement. Why not try that? □

Public going public

The British Government should revise its policy for selling off public enterprises.

THE British Government, wedded since its election five years ago to the disposal of the publicly-owned commercial corporations acquired by its predecessors, should be given pause by the latest debacle in its flirtation with the London stock markets. Last week, the sale of shares in a company called Enterprise Oil, cobbled together from the interests which the nationalized gas industry had over the years acquired in the North Sea, turned out to be a flop. Three-quarters of the shares were left with the merchant banks which had guaranteed the government the proceeds of the sale (in return for a fee). The government was further embarrassed when it came to light, late in the proceedings, that the multinational company Rio Tinto-Zinc had successfully bid for half the shares on offer. The government has promptly ruled that purchase invalid, incurring the wrath of the City of London for seeming to change the rules half-way through the sales (which allegation remains to be tested).

The following advice (which is free of charge) is offered to help the British Government to avoid similar troubles in the months ahead, when it is committed to selling off part of the nationalized automobile manufacturer (Jaguar cars), a telephone system (British Telecom) and possibly an airline (British Airways).

The motive for the sale of these public assets is twofold. The British Government holds that enterprises which could be owned privately should not remain in public hands, but also needs the cash that the sale of these assets will bring to offset its budget deficit. Thereby it has created a dilemma for itself: the public interest requires that public enterprises should be shorn as far as possible of their monopoly rights before being sold off, but the result is that they raise less cash when offered for sale. The problem is especially acute for the intended sales of the airline and the telephone company, where the government is directly in control of the monopoly rights the two enterprises will continue to enjoy. The other difficulty that has now come to light is more surprising — the government seems incapable of estimating accurately the price at which shares in public enterprises should be sold. Sometimes it underestimates what they will fetch (as with the sale of Amersham International two years ago) and is accused by its opponents of squandering public wealth. Sometimes it overestimates (as with last week's sale of Enterprise Oil) and is laughed at. The root cause of this practical problem is the ambition to sell in one operation either the whole of a publicly owned enterprise (Enterprise Oil) or roughly half of it (British Telecom).

The solution of both problems, the conflict of interest and the pricing difficulty, should however be clear. Assessing the price that shares in a public enterprise will command on the private stock markets is necessarily difficult because, while the government remains the sole owner, stockbrokers cannot "make a market". The way round that obstacle is to let shares dribble onto the market, not to unload them all at once. That, as it happens, is how the British Government is used to selling the financial securities by which it funds its own borrowing. Following such a course in the sale of public enterprises would however sharpen the conflict of interest, for then the government would be conscious that every small regulatory decision about the telephone network or the nationalized airline would affect its cash flow.

The solution is straightforward: transfer the shares in public enterprises due for sale to the equivalent of a trustee of the public interest, whose only function would be to sell shares to all who are prepared to pay the going market price for as long as the supply lasts. The only possible objection to such a policy, that minority shareholders in a company are unduly at a disadvantage, could be countered by requiring that the public trustee should scale down the voting of the shares still on his books so as to match the proportion in private hands. It is mystifying that a government that claims to believe in the market should unimaginatively be trapped by other people's rules. □

Soviet-US agreements

Reagan to review lapsed deals at last

Washington

THE Reagan Administration plans to revive six cooperative scientific agreements with the Soviet Union that the United States had allowed to languish in retaliation for the Soviet invasion of Afghanistan, the imposition of martial law in Poland and the shooting-down of the commercial Korean Airlines jet.

The announcement came in a speech last week to participants in a conference on US-Soviet relations in which Reagan attempted to walk a fine line — responding to the election-year issue of his administration's failure to ease tensions with the Soviets while at the same time reassuring the conservative faithful that he still believes the Soviet Union to be an "evil empire", as he put it in an earlier speech.

Reagan also announced willingness to re-establish cultural exchanges with the Soviet Union ("what would classical music be without Tchaikovsky", he said) and raised the possibility of a joint US-Soviet space mission intended to develop rescue techniques. An official of the National Aeronautics and Space Administration (NASA) said, however, that nothing had yet been done on "thrashing out the details" of such a mission.

The six scientific agreements that Reagan referred to have never actually expired; however, no new projects have been initiated since President Carter imposed a ban on high-level government contacts after the appearance of Soviet troops in Afghanistan in January 1980. Previously planned activities, such as joint scientific projects, symposia and information exchanges, have continued, but obviously at a much slower pace. The six agreements, all established during the period 1972-74, cover agriculture, health, artificial hearts, environmental protection, oceans and housing. A seventh existing agreement, on atomic energy, was not mentioned by Reagan in his speech or in any background information provided by the White House.

Four other agreements had earlier been allowed to lapse when they came up for renewal; these dealt with space, energy, transportation and an "umbrella" agreement for a series of workshops and studies administered through the National Science Foundation. It is considered not coincidental that these include areas in which the United States has the least to gain through cooperative ventures.

Reagan's effort to show his commitment to "better communications with the people and government of the Soviet Union" comes only a few weeks after the National Academy of Sciences cancelled a planned

visit to the Soviet Union by the academy's president, Frank Press, in the light of uncertainty over the fate of dissident Andrei Sakharov. Press had been urged not to go both by the academy's members and, quietly, by the State Department and by presidential science adviser George Keyworth. Press said that the decision to cancel his visit was consistent with the situation at the time, and that the academy has consistently maintained the importance of keeping open lines of communication with its Soviet counterparts.

Administration officials appear confident that the Soviets will go along with Reagan's proposals on the cooperative agreements. So far, the only tangible action has been on the environmental agreement; at a meeting in

Munich earlier last week, Environmental Protection Agency Administrator William Ruckelshaus met Soviet officials and discussed rejuvenating the Joint Environmental Committee, which manages projects under the agreement. Ruckelshaus was named co-chairman, a position that the United States has left vacant since 1980. (The new-found amity did not, however, prevent Soviet participants at the Munich meeting from claiming that US arms policy was damaging the environment.)

Action on the other five agreements will be taken "in the near future", according to the White House.

Reagan's proposals seem more a change of degree rather than a new initiative. Low-level scientific contacts have continued all along, even in areas where agreements have expired. Soviet and US scientists last week, for example, began a joint research cruise in the Pacific to study long-range transport of air pollutants; Soviet scientists have regularly participated in US lunar and planetary symposia, and recently presented results of the Venera 5 and 6 planetary missions.

Stephen Budiansky

Genetic engineering

Rifkin strikes at corn this time

Washington

THREATENED with becoming the next victim of Jeremy Rifkin's litigious crusade against genetic engineering, Stanford University late last week decided to postpone a planned experiment in which genetically-engineered corn plants would be grown in a test plot.

Stanford had earlier announced that the experiment, the work of Drs Ronald Davis and Virginia Walbot, was not subject to the injunction that halted the Lindow experiment on ice-nucleation bacteria (see *Nature* 24 May, p.296), and that the corn experiment would proceed this summer. After a call from Rifkin's attorney, Stanford changed its mind. The experimenters said they would voluntarily postpone the tests at least until next year.

The experiment had been approved by the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH) in 1981. The plants were to be grown on a test plot separated by a mile on all sides from other cultivated fields, and the corn tassels were to be bagged to prevent spread of the pollen. The experiment would use seed from parent plants that had been injected with a gene coding for the purple colour of Indian corn. The same experiments have been carried out in greenhouses. Unlike bacteria, corn is not self-propagating without human intervention and is not known to spread.

The injunction blocking the Lindow experiment (until a trial on the merits of Rifkin's challenge can be held) also forbade NIH from approving other

federally-funded experiments involving the release of recombinant DNA to the environment.

The ruling was, however, ambiguous on previously-approved experiments; it said NIH was enjoined from "approving or continuing to approve" deliberate-release experiments. RAC has approved one other federally-funded plant experiment (a Cornell University project involving tomatoes) and one commercial proposal, from Cetus Madison, which is not subject to the injunction.

Rifkin's legal argument is that NIH failed to prepare an environmental impact statement on the "programme" of deliberate-release experiments. If he is successful in the trial, then NIH will be forced to compile such a document, a process that NIH officials say will take at least a year.

Meanwhile, NIH director James Wyngaarden is expected to announce shortly his agreement with Rifkin's demand for "equal" treatment for commercial and federally-funded proposals that come before RAC.

The temporary injunction applied only to the latter, and at its 1 June meeting, RAC approved two deliberate-release experiments submitted by private companies. The effect of Wyngaarden's decision may be to require a formal environmental assessment for commercial proposals, the requirement that Rifkin is demanding in his lawsuit for federally-funded proposals and from which corporations have been exempt.

Stephen Budiansky

Taxation

UK budget hits research councils

BRITISH universities and research councils are still trying to count the likely cost of changed arrangements for the taxation of building works that came into effect at the beginning of June.

Under new regulations announced by the Chancellor of the Exchequer, Mr Nigel Lawson, in his budget speech in April, the cost of extensions or alterations to existing buildings (but not the cost of new buildings) is subject to Value Added Tax (VAT), now set at 15 per cent. On first estimates, the cost to the research councils could amount to £1 million in the current financial year, while universities face additional bills of between £3 million and £5 million all told.

Of the research councils, the Natural Environment Research Council (NERC) is much the worst affected. The council is at present negotiating with HM Customs and Excise over how exactly the new rules should be interpreted — it has already been awarded one favourable decision — but says that the VAT changes will cost it £100,000 even if other decisions all go in its favour; if they do not, the cost will be nearer five times as much.

NERC's chairman, Sir Hermann Bondi, has written to Mr Peter Brooke, Under-Secretary of State for Higher Education, asking for an assurance that the new VAT charges will constitute an admissible claim for supplementary grant. But Mr Brooke has not so far given any such assurance, although it is understood that discussions are being held with the Treasury over ways to help research councils. The Treasury, however, is unlikely to want to establish the precedent that public bodies can be excused VAT.

The greater proportion of NERC's VAT problems arise at one site, the new headquarters of the British Geological Survey at Keyworth in Nottinghamshire. The buildings are mostly conversions from their previous use as a college of education, and the Keyworth site alone could cost NERC up to £380,000 in VAT payments. In this event it is clear that building works would have to be delayed.

As if the VAT problems were not enough, the British Geological Survey also has serious problems with the landlord of its old London base in South Kensington. The Property Services Agency of the Department of the Environment, which administers and maintains the building, has presented NERC with a bill for £280,000 for back-payment on maintenance work, and estimates that maintenance will in future cost £350,000 a year. Since 1977, NERC has been paying just £98,000 per year for maintenance, and although some back-dated increase was expected, the amount came as a shock. In addition to the maintenance charges, rental on the building has been increased from £350,000

to £500,000 per year. NERC is still withholding the maintenance back-payment and the rent increase in the hope of being able to negotiate a compromise. But the Property Services Agency appears determined to hold out for what it describes as "reasonable and realistic" charges. The building is shortly to be transferred to the British Museum (Natural History).

In comparison with these financial headaches, the problems of the other research councils seem mild. The Science and Engineering Research Council says the VAT changes are likely this year to cost it £115,000, while the Agricultural and Food Research Council and the Medical Research Council each estimate around £200,000. Neither holds out much hope of any special allowance to recoup the cost.

Soviet refusnik

Dr Ephraim Katchalsky-Katzir, the Israeli biochemist, who was one of the leading speakers at last week's conference of the Federation of European Biochemical Societies, was last weekend unable to carry through his post-conference plans for a meeting with Jewish activists in Leningrad.

The meeting was convened by Yakov Gorodetskii, one of the activists of the unofficial Leningrad Jewish Cultural Seminar. More than 80 refusniks (Jews deprived of their jobs after applying to emigrate to Israel and then refused a visa) arrived at the appointed place, but Dr Katzir was unable to be present, being confined to his hotel room by members of the security police.

Gorodetskii and his fellow activists in Leningrad had just issued an appeal to the British Foreign Minister, Sir Geoffrey Howe, to raise the issue of Jewish emigration during his talks in Moscow with his Soviet opposite number, Mr Andrei Gromyko. This may well have focused the attention of the authorities on the group.

The current Soviet stance on emigration was spelled out in a TASS dispatch on Saturday concerning the Latvian Jew, Zakhar Zunshein, sentenced last week to three years' imprisonment for "anti-Soviet activities". Mr Zunshein's five-year campaign for a visa was described as "insulting" to the Soviet Union, and he himself was described as "inhuman", since he was ready to "abandon his parents" by going to Israel, thereby, according to TASS, contravening the Helsinki accords.

From the Soviet point of view, the Katzir incident comes as a sad postscript to a conference that had been given priority treatment. The publicity build-up in the Soviet media was considerable, including a half-page article in *Pravda* by Academician Yuri Ovchinnikov.

Vera Rich

Universities have been more vocal in their opposition to VAT changes. The University Grants Committee, among others, has been petitioning the Treasury for some relief for universities. Some older universities will escape the new VAT if their proposed alterations are to buildings listed by the Department of the Environment as being of special architectural interest, but there is nevertheless anger than universities which tightened their belts and delayed building programmes during the major squeeze on universities three years ago are now being "penalized for good financial management", in the words of Sir Alwyn Williams, vice-chancellor of the University of Glasgow.

Tim Beardsley

European Community

No decisions on research

Luxembourg

THE meeting of the research ministers of the European Community on 29 June fell a victim to the European timetable. It was too soon after the economic summit at Fontainebleau, which resolved the long-standing British budget problem, to tell how much cash was available for the ambitious programmes awaiting decision, and too soon also to be able to define priorities.

The programmes concerned include the stimulation programme, designed to help scientists to take advantage of all possible professional exchanges, whether multidisciplinary, multicultural or multinational; the BRITE programme (Basic Research in Technology for Europe), designed to help older industries to become familiar with innovative technology, and biotechnology, non-nuclear energy, radiation protection, reactor safety and fusion.

At the end of the meeting, Vicomte Etienne Davignon, vice-president of the European Commission, said that there will probably be another research council meeting this month when ministers have had time for reflection. Officials of the Science, Research and Development Directorate-general, putting a brave face on things, said that at least the past few years have seen almost unanimous support for the scientific content of new programmes, and they have been better able to manage their own budget.

Earlier in the week, however, the Communities' environment ministers had been able to decide that unleaded petrol will come a little earlier than expected. On 24 June, they agreed to the introduction of petrol without the anti-knock additive before 1989.

The British Government has already announced a planned reduction of the level of lead in petrol from 0.4 to 0.14 g per litre by the end of next year.

David Price

Technopolis

Japan's tomorrow here today

Tokyo

1984 HAS turned out to be a year of high-technology fever for Japan's local governments as they vie for new status as "technopolises", "teletopias" or "new media communities".

The names, coined from English words but already in common usage, will go to areas chosen in three large-scale government schemes for new communities centred on high-technology research or advanced telecommunications facilities. The technopolis scheme, run by the Ministry of International Trade and Industry (MITI), has already chosen twelve areas and will announce the last two this week.

The image of the technopolis, enshrined in a bill that shot through the Diet last year, is of a set of new cities, each centering on an "oasis of gleaming research institutes" and containing separate areas for non-polluting high-technology industries and for housing which, set among green fields and rivers, will seem like paradise to

Tokyo's rabbit-hutch dwellers.

The essential requirements for a candidate technopolis, as laid down by MITI, are plenty of space, a large "mother" city nearby and an easily accessible airport or major rail station that would allow the technopolitans to take day trips to Tokyo. The new cities are intended both as pace-setters for the nation's high-technology development and as magnets to move population away from Tokyo into regions where jobs for educated people have so far been scarce.

The seductiveness of the technopolis image was quickly apparent: only one of Japan's 47 prefectures failed immediately to designate a candidate region when the bill was passed. When the last of fourteen regions is chosen this week, MITI will seek to implement its promise to put the technopolis infrastructure in place by 1990. But is it all just a little bit too good to be true? Many in industry see failure, or very limited success, ahead.

The obvious problem is familiar: where will the money come from? Because the plan envisages something very much more than just a Western-style "science park", huge sums of money are necessary. Tsukuba Science City (see *Nature* 305, 378; 1983), of which the technopolises are really just smaller versions, has already cost the government more than a million million yen (compared with a gross national product of around ¥200 million million) since construction began in 1966.

Tsukuba is huge, it has around 50 institutes and 12,000 inhabitants. But although the average technopolis will be only a tenth of its size, it is unlikely that a technopolis could be built today for less than a quarter of that amount. But MITI's budget for the whole scheme in this tax year is only around ¥1,500 million — scarcely enough to buy the bulldozers. And with both central and regional governments already hopelessly in debt, there is little chance of sudden access to new funds. Instead, MITI is relying on industry and commerce to foot the bill, and is producing a package of new tax incentives and special low-interest loans which it hopes will attract high-technology companies and their research laboratories.

Industrial critics hold that this will not be enough, and that there are other serious faults in the scheme. First, fourteen technopolises is just far too many — the number chosen represents the lobbying power of prefectural governments rather than any grand plan — two or three might have been more sensible. Second, virtually all of the sites have been chosen in underdeveloped regions of the country: three on the remote northwestern seaboard, eight on the southern island of Kyushu and the most southerly part of the mainland. How can good scientists and engineers be

attracted to these regions?

In the prefectures and municipalities, enthusiasm for the second of the new schemes, "teletopia" (a brainchild of the Ministry of Posts and Telecommunications), has been even wilder. Some 99 cities are competing for teletopia status even though only ten at most will be chosen this year. Unlike the technopolitans, for whom access to airports and the ability to travel has been considered, the teletopians should not have to leave their video-screens.

The ministry plans that each teletopia should try out the fundamentals of the Information Network System (INS) (see *Nature* 305, 364; 1983). Each city will build its own local system designed to serve its special needs and those of its industries. Each will at first build its own databases, be equipped with digital circuitry to make the data quickly available, have access to the Captain system (videotext broadcast over telephone lines which provides everything from weather forecasts and train timetables to cookery and Go lessons) and cable television, and be linked to value-added network systems that will allow different kinds of computers to talk to one another.

As the system develops, electronic shopping, banking, home tutoring (including some regular schooling), medical diagnosis and video conferencing will become available, together with advanced office networks that will link word processors, facsimile transmitters and multi-purpose copiers.

At the same time, MITI is choosing seven "new media communities" to be announced at the end of this month. Already, some 135 applications have been received. The scheme is on quite a small scale and is designed to put new media, principally Community Antenna Television (CATV), to work so that information retrieval, collection and market research will be possible.

How far the three schemes will be successful remains to be seen. MITI officials, at least, say they will be pleased if the schemes are a partial success and encourage communities outside Tokyo to take up the high-technology gospel. After all, MITI regularly publishes reports entitled *A vision*... (as in *A vision for the 1980s*) and feels that supplying a long-term ideal for industry is an essential first step in steering the nation's industrial development.

Alun Anderson

Motives for mobility

EXPERIENCE with Tsukuba Science City, which is only a couple of hours from Tokyo, shows how reluctant people are to move to places without major cultural or educational facilities. Critics draw parallels between California's "Silicon Valley" and Japan's "Silicon Island" of Kyushu.

Silicon Valley has San Francisco and Stanford University nearby, and there has never been difficulty in finding bright young people for the new research and development laboratories. In contrast, Japan's microchip production is concentrated in Silicon Island, but there has been little success in moving the research staff there — virtually all design work remains firmly in Tokyo. Industry is likely to be very cautious about breaking up the massive central research laboratories put together in the 1970s.

Even so, MITI can already claim at least one major success. What the technopolis scheme has done is to awaken regions far from Tokyo to the great importance of high technology in Japan's future. It would be hard to find anyone in Japan nowadays who has not already absorbed the official view of the future: "smokestack" industries must inevitably pass to the newly industrializing nations and those already industrialized must forge ahead with knowledge-intensive industries supplying high-value-added goods.

One consequence of the acceptance of this philosophy is the relative ease with which changes in industrial structure can be made. Another is the enthusiasm of even remote regions for high-technology projects.

Alun Anderson

Nature Washington Office

STEPHEN Budiansky, Washington correspondent since 1982, is Washington Editor of *Nature* with effect from 1 July, in succession to Peter David, who will be the Washington correspondent of the *Times Higher Education Supplement* (London). Tim Beardsley is appointed Washington correspondent from the same date. □

Eastern bloc trade

Comecon sets store in science

A NEW "comprehensive programme" for scientific and technical progress is to be drafted as a result of the Comecon summit in Moscow on 12–14 June. The summit, officially the 38th (extraordinary) meeting of the Council for Mutual Economic Assistance (CMEA), held out this new programme as a basis for developing a "coordinated and, in some cases, a uniform" scientific and technical policy, to provide "the speediest solution through joint efforts" of the most important problems of science and technology on "mutually advantageous terms". A number of bilateral and multilateral cooperation agreements will in due course be drawn up to implement the programme.

Joint research ventures as a basis for integration of the socialist economies have from the beginning been hailed as an aim of Comecon. Such projects have not always proved easy. In spite of frequent official denials, there is a widespread belief in the European Comecon countries that the junior partners have had to bear far more than their fair share of the costs of the *Interkosmos* space programme. Poland, with its extensive coal deposits, has no great enthusiasm for the development of nuclear power to which the Eastern bloc as a whole is committed.

The strength of the Polish commitment to nuclear power has varied cyclically over the years, seeming weakest at periods of political "liberalization". Romania, with indigenous oil supplies, committed itself to a massive development of the petrochemical industry — Mrs Elena Ceaucescu, the wife of the president, is herself a petrochemist — and now has to import equipment to meet a capacity which has proved unnecessarily large. The underlying fear, among Comecon scientists, is that joint projects lead to the smaller members being assigned the dull and routine aspects of work, while the interesting and prize-winning aspects go largely to the Soviet Union.

In practice, the complete integration of the Comecon economies appears to have been indefinitely deferred. Speakers at the press conference which followed the summit spoke of the "harmonization" of economic policies. The real issue facing the Comecon leaders (nine party leaders and a deputy for Dr Fidel Castro) was the pooling of resources and efforts in various key areas. These include: energy production and use, including the "predominant development" of nuclear power stations and the fuller utilization of non-conventional energy sources; electronics, microprocessors, computers and robot technology, including the establishment of a "unified component base" for electronics; specialized chemicals, including plastics, chemical fibres, catalysts; "progressive technologies" for food production; and

specialized equipment for mining and civil engineering. In spite of the formal stress on science as well as technology in the official communiques, these documents only made the most oblique references to the research base, and there was no suggestion of building further joint research establishments in the tradition of the Dubna nuclear research institute or the high magnetic field/low temperature laboratory at Wroclaw.

Vera Rich

More on creationism

Washington

THE effort to repeal Louisiana's creationism-teaching law came to an end last week, for the time being at least. The state's House of Representatives voted 61–26 against the repeal motion, leaving intact the 1981 law requiring "equal time" for the teaching of evolution and creationism. The Senate had earlier approved the repeal motion by 21 votes to 16.

Repeal proponents had been counting on at least tacit support from the state's popular and flamboyant governor, Edwin Edwards, who has been trying to attract biotechnology companies to the state. In the end, however, Edwards was unwilling to help the repeal effort. Proponents say they were also hurt by a sophisticated advertising campaign that included the mailing to representatives of hundreds of telegrams, many apparently signed by persons of questionable existence, urging retention of the equal time law.

The stage is now set for a legal challenge to the law to go to trial, perhaps in January. The American Civil Liberties Union, which won a similar case in Arkansas in 1982, is arguing that the law is an unconstitutional violation of separation of church and state.

Stephen Budiansky

AIDS

Test companies chosen

Washington

THE companies selected to produce the diagnostic blood test for acquired immune deficiency syndrome (AIDS) have now been announced by the US Public Health Service (see *Nature* 14 June, p.577) and, as expected, are a mixture of the big and proven on the one hand and the small and innovative on the other. The five are Abbott, Litton, DuPont, Travenol-Genentech Diagnostics and Electro-nucleonics.

Each will pay the federal Treasury 5 per cent of net sales of the test kits, most of which will be used to test the 12 million units of blood processed each year in the United States.

The five companies were chosen from among some 20 applicants, which were

Israeli science

Ministry's fate in voters' hands

THE general election in Israel on 19 July, due to the collapse of the right coalition government earlier this year, will coincide with the second anniversary of Israel's Ministry of Science — and may settle its future. The only minister so far, Dr Yuval Ne'eman, is not only a noted nuclear physicist but also a prominent politician with hawkish views on West Bank settlements and the campaign against terrorism.

Since the right coalition government included, and to some extent depended, on the right-wing Tehiya party founded by Dr Ne'eman, some doubt whether the ministry would survive under a left coalition government. And although Mr Shimon Peres, the Labour-Alignment Party leader, has expressed a strong commitment to science, that does not necessarily imply, Dr Ne'eman's critics say, a commitment to a science ministry.

Dr Ne'eman himself is convinced that the record of the past two years has clearly demonstrated the need for such a ministry. His office, he said earlier this month in Tel Aviv, had helped with foreign relations in science and, in particular, with the setting up of bilateral international research and development agreements, which he had negotiated with his opposite numbers.

During his two years in office, moreover, Dr Ne'eman says that he has been able to help prop up the tottering finances of the universities by supporting research in university laboratories — a not inconsiderable contribution, since at least twice during the past two years, there has been a serious threat that lack of funds would force the universities to close down.

Now, the ministry has identified three main priority areas — information science

judged on their ability to produce virus, manufacture radioimmunoassays, distribute and market test kits and apply recombinant DNA techniques. Each has been provided with a 20-litre sample of Dr Robert Gallo's cell line, used to mass-produce the virus, and technical assistance from Gallo. In return the companies are subject to some unusual requirements, particularly regarding safety. Public Health Service inspectors will be allowed to examine their safety practices at any time. And the safety data that they must submit with their New Drug Applications — required by the Food and Drug Administration before the test can be marketed — will be pooled among all five companies, a departure from the normal strict secrecy that surrounds proprietary data.

Stephen Budiansky

and technology, for which a national committee has been established under the chairmanship of Dr Yehuda Kela (chief scientist of the Ministry of Communications), biotechnology (on which a preliminary report has just been completed by a team headed by Professor Ephraim Katchalsky Katzir) and technology specifically relevant to the Israeli environment (such as solar energy, solar ponds and desalination). Relatively little formal world planning of this third sector has been done so far, but all three sectors are well advanced commercially. At this stage, Ne'eman's ministry seems more concerned to give government approval (and possibly material support) to promising developments than to initiate them.

This situation is doubtless temporary. One of Dr Ne'eman's first actions as Minister of Science was to set up (under his own chairmanship) an Israeli space agency, which initially coordinated research already under way at Israeli universities, but which afterwards began to initiate projects. One such is the recent agreement with the United States for a large laser to be sited near Jerusalem for use in a bilateral programme on geodynamics (in conjunction with a satellite from the National Aeronautics and Space Administration) and which, when the satellite is not above the horizon, can also be used for meteorological and atmospheric pollution studies.

Dr Ne'eman seems a little dismayed that the Israeli press has, so far, almost entirely ignored the country's space effort. Even the political implications of Israel's application to Intelsat for a position for a geosynchronous communications satellite have made only a little impact although more than 30 overseas objections have been filed with the International Telecommunications Union, mainly from the Arab bloc.

The only "scientific" problem which the media seem keen to raise with him is the question of a possible Israeli nuclear bomb. Here, however, Dr Ne'eman's stance is unchanging — between the "doves", who would like Israel to sign the non-proliferation treaty, and the ultra-hawks, who would like to announce a definite commitment to nuclear weaponry, Ne'eman believes Israel's best hope of keeping out of a nuclear conflict lies in keeping the world guessing about its nuclear capability.

On the survival of his ministry, however, Ne'eman has recently received some coverage. Some three weeks ago the daily *Ha'aretz* acquired a leaked copy of a new report on science and technology in Israel, the first such document for fifteen years. Its predecessor, the Katchalsky report, led, among other things, to the establishment of chief scientists in all relevant ministries and also urged the establishment of a Ministry of Science, a suggestion rejected by the then prime minister Ashkol as politically inappropriate at the time.

The new report, drawn up by a commis-

sion headed by Professor Shimon Yiftah of the Technion at Haifa, a former head of the atomic energy establishment at Soreq, also strongly supports the concept of a Science Ministry, leading to the *Ha'aretz* headline: "Science Ministry will not be abolished".

Israeli innovation

Biggest science park so far

Rehovot

ISRAEL, which already has several flourishing science-based industrial parks, is to have its first fully-fledged science region. Although the project was formally approved by the government only last month, a number of companies are already operating.

The area, to be called, with just a touch of hyperbole, Region 2000, is expected to transform the Western Galilee, a sparsely populated area hitherto mainly devoted to agriculture and tourism, into a thriving centre of high-technology industry.

The scheme is the brainchild of Professor Ephraim Katzir of the Weizmann Institute, one of Israel's leading scientists and its fourth president, who has long argued that with proper planning, a large number of entrepreneurs, scientists and technicians can be attracted to the "unspoiled beauty of the Galilee". But not everyone is enthusiastic. Critics have pointed out that the existing science parks are all near well-established universities and research centres, which provide them with consultants, computers, libraries and other resources. None of these are readily available, they declare, in the Western Galilee.

This has not deterred the Ministry of Industry and Commerce, which is closely linked with the Region 2000 scheme. A ministry spokesman points out that Israel is a very small country and that the Western Galilee is only about 50 minutes by automobile from the Haifa Technion. Moreover, he adds, Sophia Antipolis, the French science city, is much further away from established universities and research centres, but has nevertheless succeeded in attracting some 60 enterprises. Situated as it is on the Riviera near Antibes, it can offer lovely surroundings and a quality of life that is not available in the older industrial regions of France.

The companies already operating in Region 2000 are chiefly offshoots of established science-based industries with headquarters in the crowded coastal plain. They include Elscint (diagnostic imaging), Elbit (computer-based systems) and Dand Iscar (jet engine compressor and turbine blades, as well as other hard-metal products).

Smaller science-based enterprises are linked to rural settlements in the area. Among them are Me'ad Computers (a software house specializing in administrative and managerial systems) at Moshav Ya'ad and Biological Industries (where advanced

Like its predecessor, however, the Yiftah commission can only recommend, not compel government action. The *Ha'aretz* headline, however encouraging to Ne'eman and his supporters, remains a piece of speculation. **Vera Rich**

tissue culture techniques are used to propagate ornamental plants free of specific pathogens) at Kibbutz Beit Ha'emek.

The existing enterprises have now been joined by two plants connected with Rafael, the Defence Ministry's weapons development authority, but only one of these will be dealing with the development and manufacture of arms — air-to-air missiles. The other, run independently under the name of Galram, will work on civilian products based on spin-offs from Rafael's military research and development.

Since existing and new enterprises in Region 2000 will need skilled manpower, high-technology vocational schools are being established to train thousands of Arab, Druze and Jewish youth; the Jewish students, who will be accommodated in a school run by the ORT organization, will include a large proportion of young people from abroad.

Economic incentives are offered to all science-based industries in Israel but, since Region 2000 is in Israel's hinterland, incentives to companies setting up there will be much more generous than those given to companies that turn to science parks in Haifa, Tel Aviv or Rehovot.

They will include cash grants and low-interest loans covering up to 75 per cent of the investment in fixed assets, 50 per cent government cost-sharing for approved research and development projects, low-interest loans to finance working capital for production, low rentals for industrial premises, a seven-year tax holiday and only 30 per cent corporate taxes. There are also special provisions to protect equity against the effects of inflation and devaluation and accelerated depreciation arrangements so that equipment can be depreciated in four years and buildings in five.

To minimize initial investment costs even further, new enterprises will be able to obtain basic administrative services from a shared services centre. These include accounting and financial services, secretarial services, production of promotional material, transport arrangements and computer and telex facilities.

Israel already exports goods to a value of more than US\$1,000 million a year in sophisticated products based primarily on local research and development and, by the end of the 1980s, according to forecasts from the Ministry of Industry and Commerce, that figure should rise to \$5,000 million. Whether or not it does will depend, in no small measure, on the success of Region 2000. **Nechemia Meyers**

Science in India discussed

SIR — Your correspondence columns have recently attracted views on Indian science from many well-intentioned scientists at home and abroad, but most contributions have failed to evaluate the plight of Indian science from the perspective of our socio-cultural value system. Scientists are not dissociated from the society in which they live and work.

Science is an intellectual, dynamic exercise based on rationality, logic and objectivity. It creates new knowledge which leads to progress in an atmosphere conducive to independence of thought. No one can claim sole proprietorship. On the other hand, our social values have been feudalistic for ages, and give precedence to personality and myth over principle and mind. Sycophancy over competence is the general rule rather than the exception in India. Science cannot be conducted in social isolation and vacuum, so finds itself on a collision course with our social values.

It was not until the late 1950s that India began to build an infrastructure for "experimental sciences" under the national science policy resolution mandate to achieve scientific self-reliance for industrial development. Despite its meagre means, India has invested heavily in this enterprise, yet in three decades, the overall return has been small. Money is being spent in the wrong places.

At the national laboratories, the quantity of instrumentation and equipment may be limited, but the quality is comparable with that of most Western laboratories. Indian scientists abroad have proved to be imaginative and productive. Yet Indian science has neither achieved international recognition nor has it found profound application in solving national problems. Even Prime Minister Mrs Indira Gandhi was recently (*Nature*, 307, 4; 1984) quoted as asking, at a meeting of directors of national research laboratories, that if there "was no hope of the situation improving, was it not time just to close them down"? This frustration is understandable. We have both qualified personnel and resources. What is lacking is the organization to guarantee an environment conducive to productivity. It is time not to close laboratories down but to rescue them through constructive changes.

The universities are in desperate shape. Many of them are fast slipping to the status of undergraduate colleges, so that many research laboratories have become places for postgraduate training. This has forced them to depend upon PhD students for research, while senior scientists are in search of PhD students rather than doing things themselves on the bench. The universities need to be revitalized, to provide sound research training and to take this burden from the research institutes.

The national laboratories should define their goals in more specific terms. A system

that assures personal independence and incentive within broad objectives needs to be introduced. Mechanisms need to be developed to monitor individual productivity, which must be rewarded in some manner within the means of the nation. Young Indian scientists established abroad should be attracted home to promote a productive scientific environment. Nothing has hurt the cause of Indian science more than the demoralization and repression of young scientists. The forces of feudalism continue to choke Indian science. The time is ripe for the political leadership to become sophisticated and assert itself.

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SIR — The recent discussions in *Nature* on the state of scientific research in India sound like the familiar arguments between Indian-based scientists, who think they are the greatest patriots, and immigrant Indians, who think of themselves as better scientists.

The first thing to realize is that science is universal and cannot be nationalized. Science and technology are relatively new in Indian society, which has nurtured arts, music and mysticism for hundreds of years in predominantly feudalistic environments. Historically, scientific and technological revolution have advanced hand-in-hand with the development of a free market economy by which the spirit of adventure and competition is usually rewarded. The adaptation of scientific research as a culture may be a painfully slow process in a country which is still uncertain as to the socio-political system that would work best in holding the country together and utilizing its limited resources.

Second, the view that Indian scientists should concentrate only on problems relevant to the country is shortsighted. Teaching and research in basic physical and biological sciences has to be maintained on a firm ground for long-term gains.

I recently spent a year at the Indian Institute of Science in Bangalore. This leading research institute maintains a university atmosphere with a great degree of intellectual freedom. My experience was not entirely discouraging. However, a general feeling of complacency about limited achievements, perhaps consistent with the fatalistic attitude ingrained in our culture, seems to be a major deterrent to progress. But there was great dynamism among certain individuals in some younger research units. A few scientists had used their past associations with good US and European laboratories to establish new techniques in their laboratories.

In this connection (see Dr Majumdar's

letter, *Nature* 308, 396; 1984), I see nothing wrong with receiving gifts from other (foreign) laboratories, which is a common practice among laboratories in the United States. But it should not be a permanent excuse for not improving the poor state of the industrial infrastructure needed to support basic research. The Indian Government, for example, has not encouraged private enterprise within the country or from abroad to compete and produce quality chemicals. In an earnest desire to improve the quality of science, however, the government has spent large sums of public money on a few national laboratories that have unfortunately been run more like bureaucratic offices than research institutes.

The hue and cry about the brain drain has really gone a little too far. In this age of highly effective communication, no brain has to be lost to a particular country. The Indian scientists working abroad can, and some do, help those in the country in small but useful ways such as with gifts of chemicals and new technical information. The fact is that our country is at present incapable of gainfully using all the immigrant brains if they were to return.

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SIR — I would be glad if you could rectify an erroneous statement made in your otherwise excellent survey of "Science in India" (*Nature* 308, 888; 1984). It is more accurate to say that Dr Kunthala Jayaraman is collaborating with the Laboratoire d'Enzymologie du CNRS at Gif-sur-Yvette, where the toxin gene has been cloned, and not, as written, in collaboration with "the CNRS laboratory near Paris". Near Paris there are some forty or fifty CNRS laboratories.

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SIR — Regardless of whether the roots of certain conceptual and practical pitfalls in the Indian system are derived from past British rule or are due to our ingrained tendency to blame the past rule when things do not work, it is clear that certain changes are necessary to eliminate impediments to efficiency and speedier progress.

What is even clearer is that time is on India's side and, given the present support and momentum, India is creating the right set of conditions to overcome these difficulties. Overall, your summary of Indian science is bound to have a positive impact on the system which has many achievements to its credit — and a working telephone system should not be too far off.

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Patterns of developing embryos

*The most important discovery this year (so far) is the recognition that genes controlling pattern formation in *Drosophila* have pieces in common with genes of unknown function in higher organisms.*

THE geometrical regularity of living things has always been a source of wonder, some of it frankly superstitious. The bilateral symmetry of leaves, the axial symmetry of many flowers and the complex geometrical regularity of insect eyes are among the common observations of the natural world which, over the centuries, have excited speculation about the nature of the invisible hand that guides the development of living things. At the turn of the century, D'Arcy Thompson (in *Growth and Form*) managed an evocative celebration of the symmetry of living things without falling into this trap. Since then, embryologists have sought in vain more objective explanations of the patterns on which living things develop. Now there is a sporting chance that the search is at an end.

The importance of the two articles appearing in this issue (pp.25 and 70) and of two just published in *Cell* is assessed overleaf by Dr Gary Struhl. What follows is meant as a plain man's incautious guide to what is certain to be a continuing source of high excitement in the months ahead, to judge both from the flood of articles now joining the queue for publication and the unmistakable inherent interest of those now published.

The new development is yet another vindication of *Drosophila* geneticists, their frequent whimsy notwithstanding. (The dominant mutation *Antennapedia*, from the Latin words for antenna and foot, gives a fruit fly a pair of legs instead of antennae, perhaps because some geneticist did not know how to decline *crux*.) The one chiefly responsible for the foundations of what has now been done is Edward B. Lewis of California Institute of Technology, who by six years ago had shown that some of the genes that control development in *Drosophila* are both functionally and physically a complex. Functionally, a mutation of one gene will lead directly to some abnormality but will also affect the expression of other genes in what appears to be a hierarchy. Physically, the genes are a complex in that they lie close together in chromosome 6 — more accurately, they lie in two clutches.

The classical analysis of the genes that control the development of *Drosophila* is nevertheless complicated enough (see E.B. Lewis, *Nature* 276, 565; 1978), simple though the animal itself may be. *Drosophila* is built on the simplest principles. The larvae and the adults consist of twelve compartments — a head

(at the front), three thoracic (chest) segments and ten abdominal segments. Each segment develops autonomously from the point in the development of the fertilized cell at which segmentation first becomes apparent. That the development is genetically controlled — not an obvious conclusion — is proved by the recognition that mutations of the development genes can make development abnormal.

Autonomous development in *Drosophila* is carried to extremes. Each of the twelve segments of a *Drosophila* larva carries inside itself a mass of tissue (called the imaginal disk) which is destined to become the corresponding segment of the adult fly at metamorphosis. Mutations of the development genes will be evident in the bizarre shape of the adult. So the destiny of the cells in each of the twelve compartments must be embodied in them

Aegean celebration

Kolymbari (Crete)

WALTER Gehring and Matthew Scott presented their groups' data on the "homoeobox" at a meeting last week at the Orthodox Academy here. The meeting, one of a biennial series of the European Molecular Biology Organization (EMBO) workshops on the genetics and biology of *Drosophila*, will be reported in more detail in forthcoming issues of *Nature*. It seems clear that there is an exciting time ahead for developmental biologists of all persuasions if indeed it turns out that the genetic control of development in vertebrates and fruit flies is similar, in principle if not in detail.

Gehring's group from Basel now has evidence for at least eleven copies of the "homoeobox" sequence in the genome of *Drosophila*, but the signs are that the final tally will turn out to be less than twenty. Struhl points out (overleaf) that some copies of the homoeobox fall outside the bithorax and Antennapedia complexes, but this observation is not discouraging. A number of speakers reported on some of the other genes concerned with regulating development on which the new powerful techniques applied to homoeotic genes are being unleashed. Of particular interest was the report of Kathryn Anderson (Tübingen) that a gene called *Toll* encodes a product that has a crucial role very early in development in establishing the dorsal-ventral polarity of the embryo.

Geoffrey North

almost from the outset.

How is this done? These days, the obvious way to find out is to learn more about the DNA of which the development genes are made. This time last year, the effort was well under way (see "Cloning the genes that specify the fruit fly", G. North, *Nature* 203, 134; 1983). Now, that effort has borne fruit. There may be two views of the outcome: either it is sheer luck that what is true for *Drosophila* seems in some sense also to hold for other organisms, or this turn of events is another illustration of the principle of parsimonious natural evolution that if you come across a useful gene (histones and globin, for example), you stick with it.

Meanwhile, there is certain to be even further interest in *Drosophila* as people scour the animal for other regulatory genes (see box). It seems at this stage to be clear that the genes now identified are concerned with the specification of the form of the organism, not with the differentiation of different functions in different tissues. (Each segment of *Drosophila* generates its own nervous tissue, for example.) So the hunt for the genes that control differentiation will continue. So will that for an understanding of how the development genes function.

Others will inevitably be more concerned with the function of the genes marked by the distinctive piece in organisms other than *Drosophila*. Organisms which are not so neatly segmented as *Drosophila* are a greater challenge, but one that can now be taken up with the techniques that have been developed in *Drosophila*. In brief, embryologists have been unexpectedly given a tool that will make the exploration of development in other organisms much more tangible than in the past, both in the cataloguing of crucial events in the course of the development of embryos and in the specification of their timing.

The importance, practical as well as abstract, of these opportunities needs little justification, which provokes an ironic chain of speculation. In Britain, the Warnock committee is about to recommend to the British Department of Health rules for the use of early human embryos in research, with a time-limit (said to be 15 days from fertilization) for the duration of such work. Hitherto, embryologists have been hard-pressed to suggest what observations might usefully be carried out. That is no longer the case.

John Maddox

Developmental biology

A universal genetic key to body plan?

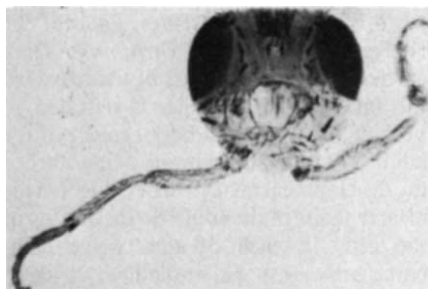
from Gary Struhl

In his treatise *Materials for the Study of Variation*¹, William Bateson reformed the sciences of embryology and evolution with a surprisingly simple idea. Recognizing that essentially all organisms are composed of orderly series of parts, Bateson proposed that chance deviations from the normal series of parts occurring in diverse animal species might provide clues about the ground rules governing development and evolutionary change. Bateson's insight effectively created a science of body pattern which has recently borne fruit in a remarkable and unexpected fashion. In a series of molecular experiments, McGinnis *et al.*² and Carrasco *et al.*³ have found that a DNA sequence common to several homoeotic genes of the fruit fly *Drosophila* is also present in the genomes of a wide spectrum of animals including annelid worms, frogs, birds, mice and man. These results suggest the exciting possibility that genes controlling the insect body plan may be homologous to a set of conserved functions controlling the body plans of a wide range of vertebrate and invertebrate animals.

The phenomenon of homoeosis was first defined by Bateson as 'the assumption of one member of a meristic series of the form or character proper to another member of the series'. 'Homoeotic mutations' in insects characteristically result in the apparent substitutions of one or more segments by segments normally found elsewhere along the body axis. For example, in silk worms homozygous for the *E^N* mutation, all of the abdominal segments of the caterpillar develop incorrectly as leg-bearing thoracic segments⁴. Homoeotic mutations therefore appear to disrupt a set of genes which normally act to control the particular form and cell pattern characteristic of each segment. The existence of such genes argues that the segments composing an insect's body are interchangeable, their diverse forms and patterns representing elaborate variations on a common theme.

Beginning with E.B. Lewis's initial studies of the bithorax complex of *Drosophila*⁵⁻⁷, the evidence that homoeotic genes might contain an evolutionarily conserved component has grown progressively more compelling. Having discovered that several homoeotic genes with closely related roles formed a tightly linked cluster or gene complex, Lewis proposed that these genes might have arisen as tandem duplications of a single ancestral gene. More recently, Tom Kaufman and co-workers have provided evidence that several other homoeotic genes are similarly organized in a second cluster⁸, the Antennapedia complex,

again suggesting a common origin for different homoeotic genes. The isolation of both the bithorax⁹ and Antennapedia^{10,11} complexes by recombinant DNA techniques immediately allowed for direct comparisons of their molecular properties and in turn led to the finding that both complexes have very similar structures and transcriptional properties. For example, both complexes are very large (200–400 kilobases) and give rise to unusually long transcripts (up to 100 kb) which are then processed to yield a number of different



Legs replace antennae on the head of a *Drosophila* that has a mutation within a homoeotic gene of the Antennapedia gene complex. Some homoeotic genes of *Drosophila* have a short 'homoeo box' sequence that is also found in the genomes of worms, frogs, mice and man.

messages. In addition, their transcripts first appear during the same early stage of embryogenesis (the cellular blastoderm stage) and are tightly localized to the primordia of particular segments¹²⁻¹⁴, as predicted by previous genetic studies⁷. These close parallels suggested that the two complexes might themselves be homologous gene systems having a common evolutionary origin.

In light of the many genetic and molecular similarities between genes of the bithorax and Antennapedia complexes, it may come as a surprise that no clear evidence of sequence homology was detected until the recent studies of McGinnis *et al.*¹⁴ and Scott and Weiner¹⁵. The explanation appears to be that there are only a few regions of DNA homology within and between these complexes, and these regions are quite small (180 base pairs). The principle results obtained in these studies can be summarized as follows. A common highly conserved sequence — the 'homoeo box' — is found near the 3' end of the mature transcripts of at least two homoeotic genes of the Antennapedia complex and three homoeotic genes of the bithorax complex. This same sequence is also found at the 3' end of the transcript of a sixth gene (*fushi tarazu*) which lies in the middle of the

Antennapedia complex, though this gene has a rather different role in the process of segmentation than the homoeotic genes surrounding it. Finally, only one or a few other homoeo box sequences have been detected in the rest of the *Drosophila* genome. Thus, the seven or so homoeo boxes found in the *Drosophila* genome comprise a coherent family of highly conserved DNA sequences most of which map to known homoeotic genes within the bithorax and Antennapedia complexes.

McGinnis *et al.*², Scott and Weiner¹⁵ and Laughon and Scott¹⁶ (see page 25 of this issue) have determined the sequences of homoeo boxes found in both complexes. All have a common open reading frame encoding a highly conserved and extremely basic polypeptide. Furthermore, as reported on page 70 of this issue by Shepherd *et al.*¹⁷, this polypeptide shows some similarity to polypeptide sequences encoded by two yeast mating-type genes and possibly to the DNA-binding domains of a few prokaryotic regulatory proteins¹⁶. As discussed by both groups, these results suggest the possibility that the homoeo box encodes a conserved DNA-binding moiety which is tagged onto a diverse set of homoeotic gene products. This suggestion fits nicely with the early proposal of Garcia-Bellido¹⁸ that the principal role of homoeotic genes such as those of the bithorax complex would be to regulate the expression of large batteries of 'realizator' genes, thereby specifying segment-specific patterns of cell differentiation.

The discovery of common homoeo boxes restricted to the two homoeotic gene complexes provides direct evidence that there is at least one evolutionarily conserved component of homoeotic genes in *Drosophila*. This conclusion becomes all the more significant in light of the dramatic discoveries of McGinnis *et al.*² and Carrasco *et al.*³ that small numbers of homologous homoeo box sequences are present in the genomes of a wide spectrum of segmented animals ranging from worms and insects to frogs, chickens, mice and man.

Perhaps the most exciting way to interpret these results is in terms of the apparent correlation between segmentation and homoeo boxes. All of the animals that appear to contain homoeo boxes pass through a developmental stage when the body is composed of a linear series of segmental units. For example, all vertebrate embryos form a reiterated pattern of somites from which the basic meristic organization of the skeleton, nervous system and musculature is ultimately derived. We know already that homoeo boxes in insects are part of the mechanism that controls the diversity of segment types. Hence, their homologues in vertebrates might serve a similar function in controlling diversification of the embryonic somites.

If correct, this possibility may prove to be a major breakthrough in understanding vertebrate development because it means that many of the principles which govern

the development of insect segments might have direct counterparts in vertebrate systems. For example, segments in insects are developmental compartments^{18,19}; each arises as a group of blastoderm cells all of whose descendants form a precisely defined portion of the larval and adult body. Segments are also the precise realms of action of particular homoeotic genes such as those of the bithorax and Antennapedia complexes. This intimate correlation between cell lineage and homoeotic gene action has led to the proposal that the insect body pattern is initially determined by the irreversible activation of particular homoeotic genes in the founder cells of each segment. Accordingly, all the descendant cells inherit the same pattern of active homoeotic genes and hence form a polyclone with a common 'genetic address'²⁰.

The discovery of vertebrate homoeo boxes now allows us to apply this 'selector gene' or 'compartment' hypothesis directly to vertebrates because it predicts that at least some of the vertebrate homoeo box sequences will belong to homoeotic genes like those of the bithorax and Antennapedia complexes. Thus, we can ask whether these genes function in discrete subsets of somites, and even whether somites themselves are developmental compartments. Depending on the success of this approach it may be possible to extend the analysis to earlier developmental events, such as those responsible for specifying the correct number or sequence of segmental units in the early embryo. Here again, much of what is already known from genetic studies in insects may be directly applicable to vertebrates. Indeed, we can now test whether at least some of the many genes which govern these processes in *Drosophila* may have homologues in vertebrate systems.

Despite having described many examples of homoeotic variations in man and other vertebrates, as well as in arthropods,

Bateson stressed that the meristic organization of vertebrates and invertebrates might represent analogous rather than homologous solutions to organizing the body plan. Indeed, he argued strongly from embryological data that arthropod segments and vertebrate somites had evolved independently. The existence of homoeo boxes in a wide spectrum of segmented animals does not, in itself, resolve this issue, though it does argue strongly for at least one close evolutionary link. What is perhaps more important, however, is that the discovery

of homoeo boxes provides a precedent as well as a method for looking for homologous functions controlling the development of diverse animal forms. By identifying, comparing and contrasting the roles of such functions we may well gain insights into the general principles which govern development and evolution. □

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Atmospheric chemistry

Henry's Law confounded

from Peter Brimblecombe

KNOWLEDGE of the solubility of gases in aqueous solution is required for a wide variety of environmental calculations. These include the estimation of the rate of gas exchange across the air-sea interface and the efficiency of scavenging by rainfall. Ammonia is the most abundant alkaline gas in the atmosphere, so some of the interest in its atmospheric chemistry is related to concern over acid rain. For a number of years authors have drawn attention to a surprising disagreement between the predicted concentration of ammonia in rain and that which is actually measured. Lau and Charlson (*Atmos. Environ.* 11, 474; 1977) suggested that the discrepancy could be as large as three orders of magnitude, implying that the solubility of low concentrations of ammonia in water might be very much lower than originally thought. Recently, Ayers *et al.* (*Tellus* 36B, 85; 1984) have returned to this nagging problem.

Gas solubilities are frequently published in terms of the constant of proportionality which relates the partial pressure of the gas to the amount dissolved. This derives from a relationship published by William Henry in *The Philosophical Transactions of the Royal Society* of 1803. One of Henry's concerns had been to disprove Dalton's Law of Partial Pressure. Much to his own surprise, he actually confirmed it and at the same time discovered the law which now bears his name. Henry's Law holds in many dilute solutions and is considered to be obeyed in all solutions at infinite dilution.

The history of deviations from Henry's Law is a long one. Deviations in the behaviour of ammonia were observed as early as 1860 when Roscoe and Dittmar found that its aqueous solutions did not obey Henry's Law below 60°C. The reason for this discrepancy arises from the equilibria that follow the dissolution of ammonia in water. That problem is easy enough to resolve: once the effect of hydrolysis is taken into account, the solubility of ammonia agrees with the theory.

The large errors in the Henry's Law con-

stant of ammonia implied by atmospheric measurements prompted Hales and Drewes to redetermine the solubility of ammonia in aqueous solution (*Atmos. Environ.* 13, 635; 1979). Their measurements were made at extremely low concentrations where behaviour should follow Henry's Law very closely. In distilled water there was broad agreement with earlier determinations, but the experiments showed an unexpected dependence of ammonia solubility on carbon dioxide concentration that is difficult to explain. And there seem to be other very odd things about the dissolution of ammonia. P.W. Balls (PhD Thesis, Univ. East Anglia; 1980) made a number of measurements of the rate of dissolution of ammonia in aqueous solution. The results, he decided, could only be explained by assuming that the liquid phase boundary layer had a seemingly impossible negative resistance.

While experiments of these types leave us with the impression that there is much that is still very confusing about dilute aqueous solutions of ammonia, they also show that there is nothing to suggest that the older values for the Henry's Law constant of ammonia are wildly in error; at least nothing as large as the thousand-fold difference implied by the concentrations of ammonia found in rainwater.

In an effort to resolve the problem, Ayers *et al.* have repeated the atmospheric measurements, taking particular care to determine the ammonia concentration in simultaneously collected air and rainwater samples, although the low atmospheric ammonia concentrations necessitated sampling times that were often considerably in excess of the duration of rainfall. Once again the relationship between gas- and liquid-phase concentrations was not described by Henry's Law and again it was the concentrations in the liquid phase that were lower than expected. The authors leave us with two possible explanations: either the Henry's Law constant obtained in laboratory studies is inappropriate in the atmospheric context; or the gas and liquid droplets were not in equilibrium, despite

1. Bateson, W. *Materials for the Study of Variation Treated with Especial Regard to Discontinuity in the Origin of the Species* (Macmillan, London, 1894).
2. McGinnis, W., Garber, R.L., Wirz, J., Kuroiwa, A. & Gehring, W.J. *Cell* 37, 403 (1984).
3. Carrasco, A.E., McGinnis, W., Gehring, W.J. & de Robertis, E.M. *Cell* 37, 409 (1984).
4. Tazima, Y. *The Genetics of the Silk Worm* (Logos, London, 1964).
5. Lewis, E.B. *Cold Spring Harb. Symp. quant. Biol.* 16, 159 (1951).
6. Lewis, E.B. *Am. Zool.* 3, 33 (1963).
7. Lewis, E.B. *Nature* 276, 565 (1978).
8. Kauffman, T.C., Lewis, R. & Wakimoto, E. *Genetics* 94, 115 (1980).
9. Bender, W. *et al. Science* 221, 23 (1983).
10. Garber, R.L., Kuroiwa, A. & Gehring, W.J. *EMBO J.* 2, 2027 (1983).
11. Scott, M.P. *et al. Cell* 35, 763 (1983).
12. Hafen, E., Levine, M., Garber, R.L. & Gehring, W.J. *EMBO J.* 2, 617 (1983).
13. Akam, M.E. *EMBO J.* 2, 2075 (1983).
14. McGinnis, W., Levine, M.S., Hafen, E., Kuroiwa, A. & Gehring, W.J. *Nature* 308, 428 (1984).
15. Scott, M.P. & Weiner, A.J. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
16. Laughon, A. & Scott, M.P. *Nature* 310, 25 (1984).
17. Shepherd, J.C.W., McGinnis, W., Carrasco, A.E., de Robertis, E.M. & Gehring, W.J. *Nature* 310, 70 (1984).
18. Garcia-Bellido, A. *Ciba Fdn Symp.* 29, 161 (1975).
19. Lawrence, P.A. *Cell* 26, 3 (1981).
20. Garcia-Bellido, A., Lawrence, P.A., & Morata, G. *Scient. Am.* 241, 102 (1979).

relatively long contact times between the liquid droplets and the gas phase.

There seems to be another possibility, however, namely that the ammonia concentrations measured are not representative of the gas-phase equilibrium concentrations. Bowersox and de Pena (*J. geophys. Res.* **85**, 5614; 1980) have emphasized that ammonia is so soluble under the pH conditions encountered in atmospheric precipitation that the gas partitions almost completely into the liquid phase. This means that measurements of gas-phase ammonia, which have mostly been made when it is not raining, may vastly exaggerate its concentration during rainfall. In our own approach, George Dawson and I have chosen to use a partition equilibrium to allow for this; we predict that pH should have no effect on dissolved ammonia concentration in rainwater, while the conventional use of Henry's Law implies an increase with decreasing pH.

What appears to be significant is that the discrepancy between predicted and measured concentrations of the dissolved gas in rainfall arises with a gas whose solubility is extremely high; so high, in fact, that under normal cloud conditions it will reside predominantly in the liquid phase. This is true of very few gases, because the volume of liquid water in clouds is usually a million times less than that of the gas phase. Hydrogen chloride, hydrogen peroxide, nitric acid and sulphuric acid are other important atmospheric trace constituents that are soluble enough to partition almost completely into the liquid phase. It remains to be seen whether their concentrations in rain are also much lower than those predicted by the direct application of Henry's Law. □

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Plant ecology

The sex life of primroses

from A. J. Richards

A BLAZING controversy is best fuelled by a lack of good data. Crosby's discovery of a self-pollinating self-fertilizing primrose was accompanied by the contentious claim that the variant would spread rapidly through southern England because of the advantage that selfing seems to offer (based largely on observations of laboratory plants) in terms of the number of seeds produced per plant. During the subsequent decade it became evident that no such takeover was occurring; if anything the variants were retreating. Walter Bodmer (now Director of Research for the Imperial Cancer Research Fund) noted that no Somerset primrose population had more than 80 per cent of the variant form, and in most the proportion was much less (*Heredity* **12**, 363; 1958). This raised the question of the real extent of cross-fertilization in the variants. It is on this point that good data (but not futile arguments) have been lacking but are now provided by Piper *et al.* on page 50 of this issue.

The variant primroses are homostyles: their stigma-bearing styles are all of the same length and surrounded by the anthers, in contrast to the usual heterostyle primroses, composed of 'pins' — in which the stigma is above the anthers — and 'thrums', in which it is below them. Bodmer argued that despite the proximity of the stigma and the anthers in homostyle primroses, up to 80 per cent of outcrossing onto homostyles could occur during the 2.5 day protogynous phase of each flower, when its stigma is receptive but its anthers are not ready to release pollen. This remarkable statement was based entirely on the proportion of pin seedlings (*ss*) arising from homostyle heterozygotes (*S^hs*)

cultivated in a plot containing only homostyles, some of which were homozygotes *S^hS^h*. A deficiency of pin seedlings from the expected 1:3 was explained by the assumption of high levels of outcrossing with *S^h* pollen.

If Bodmer was correct, such high levels of outcrossing would discourage the spread of the *S^h* chromosome in wild populations. Crosby's model had assumed that homostyle mothers were entirely selfed, and that homostyles also randomly competed with thrums as male parents.

Crosby countered Bodmer by showing that controlled selfs from known homostyle heterozygotes, *S^hs*, produced a deficiency of pins, apparently due to their lower viability (*Heredity* **13**, 127; 1959). He also produced estimates of outcrossing from artificial populations with open pollination, and from seedlings derived from wild populations, which independently suggested outcrossing rates onto homostyles of between 5 and 10 per cent, sufficiently low to encourage the spread of the homostyle chromosome. Crosby suggested that the reason why the homostyle chromosome did not spread was not because of high levels of outcrossing, but because the viability of homostyles was only 65 per cent of that of heterostyles.

The trouble is, neither Crosby nor Bodmer studied outcrossing rates onto homostyles of primroses in the wild; they did not investigate pollen loads on stigmas; they did not use unbiased marker genes, independent of the breeding system; they did not even test the supposedly greater fecundity of wild homostyles. In the absence of such basic facts, Crosby's elegant model is no more than stimulating,

and Bodmer's progenies irrelevant to the field situation. Now, after a lapse of a quarter of a century, John Piper, Brian Charlesworth's student at the University of Sussex, has produced field data relevant to this much-discussed problem.

At last we know that homostyles in the field really do produce more seed than heterostyles: 65 per cent more per plant, or 62 per cent more per flower, on average. Whether this seed is as viable, or the seedlings as fit as those from heterostyles remains to be determined. Is it possible that the more plentiful better-filled capsules of the homostyle even constitute a disadvantage in mixed populations, by presenting a more rewarding target to predators?

At last we know that the load of selfed pollen on homostyle stigmas is massively (35 times) greater than that of thrum pollen on pin stigmas — enough, it would seem, comfortably to account for very high levels of selfing. It is worth noting, however, that the average pollen load on the homostyle stigma is 20 times greater than that needed to fertilize all the ovules in a flower. If we accept that thrum-type pollen loads onto pins can be used as an estimate of crossed loads onto homostyles, then there is enough crossed pollen to fertilize about 60 per cent of homostyle ovules. If this crossed pollen reaches the stigma while the flower is still in the protogynous phase, as Bodmer suggested, it may outcompete the large quantities of pollen which are automatically selfed onto the homostyles as soon as their anthers mature.

There is another faint worry; many visitors to *Primula* are pollen feeders and the homostyle and the thrum present their pollen more obviously than does a pin flower and so may be more attractive to visitors. Thus, comparisons with pin flowers might underestimate the amount of cross-pollination that takes place onto homostyles.

Piper *et al.* also provide independent gene markers to estimate the actual genetic effects of the different breeding systems. Although only two markers are used, the results are remarkably consistent with each other. (There is an unspoken assumption that the allozyme bands in these systems are allelic with respect to each other, and are selectively neutral.)

Populational substructuring is caused by pollen and/or seed travel which is limited in extent and thus non-random within a total population. However, Piper *et al.* discount this as a factor that might lead to an overestimate of inbreeding. In the related, and much more efficiently pollinated, cowslip (*P. veris*), gene travel within the population is distinctly limited (Richards, A.J. & Ibrahim, H. in *The Pollination of Flowers by Insects* ed. Richards, A.J., Academic London; 1978). It would be surprising if this were not also the case in the primrose. Thus, the estimates of selfing reported by Piper *et al.* may be exaggerated. It is notable that the heterostyle figures for *s* do

not reach zero (0.06 and 0.20), although the heterostyle primroses are probably totally self-incompatible, and completely cross-fertilized. If limited gene flow has resulted in this slight inbreeding, then perhaps the homostyle s figure of 0.99 is also too high. After all, it is levels of selfing, not levels of inbreeding, that are being investigated. It is always tempting to use isozyme data from populations to characterize the breeding system, not the genetic effects of the breeding system, but this can lead to circularity.

In the case of a few organisms, an irresistible invitation to investigate the complexities of organic evolution seems almost God-given. Like *Drosophila*, *Cepaea*, *Papilio* or phage, the pin/thrum/homostyle system in *Primula* has attracted a string of evolutionary geneticists; their names read like a roll of honour for the science: Darwin, Bateson, Gregory, Haldane, Fisher, Mather, Ford, Dan Lewis, Darlington. Although there is still a great deal more to learn, it now seems that Crosby is largely vindicated; homostyles do largely self, and they do set more seed than heterostyles. So why have they only established themselves in Somerset and the Chilterns and why don't they succeed better there? Is it a lack of genetic variability? Is it inbreeding depression? The answer may be far more mundane, as was suggested by Crosby some years ago. Slugs and snails browse on primrose flowers, at the mouth of the tube. Grazed pins become male, grazed thrums become female but grazed homostyles are neutered. □

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100 years ago

HAVING noticed some time ago a number of letters in *Nature* on the above subject, I venture to publish an instance, which came under my own observation last month, of extraordinary intelligence in a rat. I was standing in the doorway of a large shed, the further end of which had been partitioned off with bars to form a fowl-house, when I was attracted by a gnawing and scraping noise; turning round I saw a rat run from a large dog-biscuit which was lying on the floor, and pass through the bars. Being curious to watch if he would return. I kept quiet, and presently saw a well-grown specimen of the "common brown rat" (*Mus decumanus*) come cautiously forward, and after nibbling for a short time at the biscuit, drag it toward the bars, which are only two inches apart, and would not allow the biscuit to pass. After several unsuccessful attempts he left it, and in about five minutes returned with another rat, rather smaller than himself. He then came through the bars, and, pushing his nose under the biscuit, gradually tipped it on edge, rat number two

Astronomy

Galaxy evolution at late epochs

from M.S. Longair

ONE of the major growth areas of extragalactic astronomy over the last few years has been the comparison of the properties of distant galaxies with equivalent samples of nearby galaxies. The outcome is an intriguing jigsaw puzzle, a new and surprising piece of which has come from the comparison of the properties of galaxies in clusters at different distances, and consequently at different cosmological epochs, reported on page 31 of this issue of *Nature* by H. R. Butcher and A. Oemler. The surprise is that a great deal of star formation is still going on in a cluster so late in the life of the Universe.

The cluster, Cl1447+2619, has a modest redshift of 0.38, at which the Universe is about three quarters of its present age. Nevertheless it shows signs of vigorous star formation, very much more so than is observed in similar irregular clusters at the present epoch. The previous work of Butcher and Oemler had suggested such a phenomenon on the basis of the colours of the galaxies in the cluster; it is now confirmed by multi-object spectroscopy using the Kitt Peak 4-metre telescope. Interpreting these data as evidence that the cluster is still in the process of settling down to become a normal irregular cluster, Butcher and Oemler speculate that, if this phenomenon is common to all galaxies, there must be a great deal of star formation going on in galaxies at these and greater redshifts.

pulling vigorously from the other side; by this means they finally succeeded in getting a four-inch biscuit through a two-inch aperture. Not feeling pleased that my dog's biscuits should be used as food for rats, I threw a hammer at them and picked up the biscuit.

A REMARKABLE shower of black rain fell here in the neighbourhood last Sunday, the 22nd inst. The forenoon had been fine, though somewhat hazy, but about 3.30 p.m. heavy cumuli formed to north and north-west. Gradually a dense mass of cloud and haze came from the northward, presenting a lurid, threatening aspect, and it became so dark that one could not read a book indoors. At 4.30 rain began to fall, at first a few drops, and soon after a heavy downpour. When this commenced I noticed a number of black objects floating in the air, which I at first took to be flies or winged ants, but they rapidly increased in number, and on looking at them more closely I found them to be particles of soot, on an average about the size of the common fly. Their number was so great that it appeared for ten minutes to be snowing black, the descent of the blacks being slow, like that of snowflakes. After it had rained heavily for fifteen minutes, these "blacks" ceased and the air became lighter, but the rain continued for another hour, and altogether I measured 30 inches in my gauge.

From *Nature* 30, 3 & 10 July 1884.

A variety of different lines of investigation have recently been followed in the study of questions of this nature. In one approach, very large samples of very faint galaxies have been studied to find out whether the counts of these objects are consistent with the predictions of different world-models. In this type of work, there is essentially no redshift information about the galaxies and so one is simply taking averages over very large numbers of the faintest galaxies observable. The work of T. Shank and his colleagues at Durham University, and of D.C. Koo and R.G. Kron while at Berkeley, has shown that the galaxy counts are in reasonable agreement with the predictions of uniform world-models at red wavelengths, although there is some evidence for more blue galaxies than expected when the counts are made in blue light. This work has suggested that very strong evolutionary effects which occur in all galaxies can be excluded.

Other studies have concentrated upon much smaller samples of galaxies but have compensated for this by using objects which have measured redshifts, particularly the most luminous galaxies with very large redshifts. An example of this approach is the study of the brightest galaxies in rich clusters, one of the prime motives being to estimate the deceleration parameter of the Universe since these galaxies appear to be rather good standard candles. The problem with this type of study, which has extended to redshifts of about 0.7, is that only the brightest cluster members can be studied in detail. At a redshift of 0.5, similar to that at which Butcher and Oemler find strong differences between their cluster and nearby examples of the same type of cluster, the redshift-magnitude relationship shows little evidence of deviation from that predicted.

An alternative approach, taken by Lilly and myself, is to study the stellar populations of the giant elliptical galaxies that are strong radio sources. The advantage is that these can be selected in a manner which is not biased by optical selection criteria; moreover, the galaxies have strong narrow emission-line spectra from which redshifts of up to 1.6 have been measured. In our analysis of the colour-redshift diagrams for these galaxies we find evidence both for evolution of the underlying old stellar population and for an excess of blue light which we interpret as evidence for a young stellar population in these galaxies. However, we find these effects occur at redshifts greater than about 0.5.

Finally, on the observational side, it has been established for many years that the populations of galaxies with active nuclei, such as radio sources and quasars, exhibit

very strong evolution of their properties with cosmic epoch, even over the redshift range of 0–1, in the sense that such objects were much more common when the Universe was half its present age. Thus, there must be some major changes in the properties of the host galaxies over this time period.

Does all of this add up to a consistent picture? It is possible to give a guardedly positive answer on the basis of some suggestive trends emerging from the separate approaches.

That active star formation is going on in certain types of galaxies at relatively late epochs in the Universe seems incontrovertible. There must therefore be gas and dust in these systems. In a number of cases, this activity can be ascribed to interactions between galaxies. The IRAS satellite has very convincingly shown that the most luminous galaxies in the far-infrared are the interacting galaxies and that the luminosity is probably due to heated dust. This is the signature of 'star-burst' galaxies in which stars are being formed. In the case of the radio galaxies, we suggest that the presence of the young stellar population is due to interactions between galaxies which provide the fuel to generate the activity observed in the active nucleus. Some support for this hypothesis comes from the fact that a considerable fraction of active galaxies and quasars seem to have companions. Since, however, the counts of galaxies do not show major departures from the predictions of uniform models of

the Universe, this type of phenomenon cannot be all that common in the Universe.

It is not clear at this stage whether there is any relationship between any of these ideas and the observations of Butcher and Oemler. That star formation is proceeding in a very large fraction of galaxies in the cluster Cl1447+2619 is incontrovertible. Its interpretation is less obvious. It is conceivable that interactions between galaxies in the cluster are important but it is not obvious why all irregular clusters should not show the same phenomenon. As the authors themselves suggest it may well be that the cluster is significantly younger than the system observed nearby. According to the adiabatic theory of cluster formation, which has had some significant successes in recent years, clusters form late in the Universe; Cl1447+2619 may be an extreme late-developer. The existence of such systems is clearly of the greatest cosmological interest and we need to know how common they are.

These observations give a clear picture of what can now be achieved in the study of normal galaxies at epochs earlier than the present. The ways forward are self-evident but they are very demanding observationally. They do, however, bring astrophysical studies of galactic and cluster evolution significantly closer to a real observational science. □

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Gene regulation

What controls the transcription of immunoglobulin genes?

from Robert P. Perry

EFFORTS to understand the regulation of eukaryotic genes have resulted in the identification of an impressive, sometimes bewildering, array of DNA sequences (regulatory elements) that can influence the transcription of genes into RNA. Many of these elements act in *cis* and are believed to affect DNA topology, producing local conformations that selectively allow or restrict access of RNA polymerase to the DNA template, or that facilitate selective opening of the double helix at the site of transcriptional initiation. It is becoming increasingly apparent that evolution has endowed different classes of genes with distinctive combinations of different regulatory elements. One good example which has received considerable attention lately is the group of genes that encode the immunoglobulins. These genes are novel in that the regulatory elements are widely separated in the germ-line genome but are brought together by somatic recombination in cells of the B-lymphoid lineage. Moreover, there is evidence that the

function of one of these elements may depend upon an interaction with a *trans*-acting factor that is differentially active over the course of lymphocyte maturation. Two *cis*-acting elements have already been identified in immunoglobulin genes: now two laboratories have independently identified a third, that may also participate in the tissue-specific transcription of the genes (Parslow, T.G. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 2650; 1984 and Falkner, F.G. and Zachau, H.G. *Nature* **310**, 71; 1984).

In general, there are two broad categories of *cis*-acting regulatory elements. Elements near to the initiation site, frequently termed promoters by analogy to their prokaryotic counterparts, may consist of a short sequence (like the TATA box) which apparently helps define the start site for RNA polymerase II, as well as sequence motifs further upstream that are presumed to regulate the efficiency of polymerase loading. The second type of element, the more remotely located

elements, typified by the so-called enhancers, can exert their effect over considerable distances when located on either side of the initiation site. The extent to which a particular set of elements affects DNA topology may be determined by several extrinsic parameters including the extent of methylation of the DNA, the ionic environment, the complement of chromosomal structural proteins and certain locus-specific *trans*-acting factors, usually presumed to be proteins.

The two principal gene segments that together form an immunoglobulin gene are the variable (V) segment and the constant (C) segment, widely separated on the same chromosome in the germ-line DNA. The regions encoding the V portions of both the heavy-chain and κ light-chain genes are, in general, transcriptionally silent in their unrearranged context in the germ line and become active only upon rearrangement, whereas the regions containing the constant portions of these genes (J_H-C_H or J_K-C_K) are transcriptionally active in the germ line as well as in the rearranged context. A relationship between this transcriptional behaviour and DNA topology is suggested by the fact that in plasmacytoma cells, the C_K locus, whether rearranged or not, is undermethylated and relatively sensitive to digestion by the enzyme DNase I, whereas unrearranged V_K genes are usually methylated and in DNase I-resistant chromatin. The transcriptional competence of the constant regions is thought to be related, at least in part, to the presence of enhancer elements which are located in the introns separating J_H and C_H or J_K and C_K . These elements, which can enhance transcription of immunoglobulin and other genes when placed on either side of the transcriptional initiation site, are preferentially active in lymphoid cells. They have been mapped by deletion analyses to regions spanning about 200 nucleotides. Furthermore, the chromatin of the enhancer regions contains sites that exhibit DNase I and restriction endonuclease hypersensitivity in lymphocytes but not in non-lymphoid tissue, thus suggesting a relationship between enhancer activity and alteration of the chromatin structure.

The foregoing observations are consistent with a model in which the enhancer propagates a signal upstream to a region which permits proper transcriptional initiation by RNA polymerase II. In its most general formulation, the model does not specify whether this signal involves the

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polymerase itself, as would be the case if the enhancer segment served as a very efficient entry site for initial polymerase binding, or whether the enhancer acts solely via its influence on the conformation of an upstream polymerase binding site. The latter interpretation presumes the existence of a promoter-like element upstream of the transcriptional initiation site, which would be susceptible to *cis* regulation by the enhancer. It is already known that *V* genes have TATA box elements in the necessary upstream position; now Parslow *et al.* and Falkner and Zachau (*op.cit.*) have identified a second set of upstream sequences that may be essential for promoter function.

An alignment of the sequences that flank the transcriptional initiation site of a relatively large number of mouse and human *V_κ* genes as well as the mouse *V_λ* genes has revealed a very highly conserved octanucleotide, ATTTGCAT, located about 60–80 nucleotides upstream of the initiation site. Moreover, gene transfer experiments in which deletion mutants of a functional κ gene were integrated into the genomes of plasmacytoma cells that were not producing immunoglobulin have demonstrated that the region containing this octanucleotide is essential for effective transcription (Falkner & Zachau, *op. cit.*). Remarkably, the *V_H* genes do not contain this sequence but its inverted complement (ATGCAAAT) at precisely the same location relative to their transcriptional start site. These octanucleotide elements may have a role analogous to the various similarly located sequences that have been shown to be important for the transcription of other genes; together with the TATA box, they may comprise the functional promoters of immunoglobulin genes. Although a more narrowly defined set of mutants will be needed to establish whether the octanucleotide is a critical part of the promoter, its almost ubiquitous occurrence in all *V* genes makes it a most attractive candidate. Indeed, for this gene family, its conservation greatly exceeds that of the TATA box. Interestingly, there is a perfect ATTTGCAT sequence in a region of the *J_H-C_μ* intron where some of the germ-line (sterile) heavy-chain transcripts are initiated and an almost perfect inverted sequence (ATGTAAAT) upstream of the site where germ-line κ transcripts are initiated. It is conceivable that these sequences help define the 'pseudopromoters' that are used in the generation of germ-line transcripts.

The difference in transcriptional competence between unrearranged *V* and *C* regions suggests that some type of regulatory hierarchy between enhancer and promoter elements is maintained throughout the course of B-lymphocyte development. The recent finding of a heavy-chain gene that presumably lacks its normal enhancer and yet still produces heavy-chain mRNA has been interpreted to mean that the enhancer function is not obligatory for

expression (Wabl, M.R. & Burrows, P.D. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2452; 1984). However, it is difficult to evaluate the significance of this observation because the gene has not yet been sequenced; conceivably the enhancer function has been taken over by some surrogate sequence element, just as the pseudopromoters can substitute for the proper *V*-region promoters.

The enhancer element seems to require a tissue-specific *trans*-acting factor, the activity of which is subject to induction by external stimuli during the early (pre-B-cell)

maturation stages and becomes constitutive in later (B-cell and plasma-cell) stages (Nelson, K.J. *et al. Nucleic Acids Res.* **12**, 1911; 1984). The promoter may also be regulated by *trans*-acting factors. If so, such factors might be responsible for the increased rate of transcription that is characteristic of terminally differentiated plasma cells. □

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A solar coronal mass ejection observed in white light by the Solar Maximum Mission coronagraph. The axis of solar rotation runs from the upper left (north) to the lower right; the Sun-centred occulting disc has a radius of 1.6 times the radius of the Sun. The outward velocity was 200 km⁻¹. The ejection is typical of 65 observed during the Solar Maximum Mission between March and September 1980 in that it has a three-part structure: an inner bright loop sometimes seen in H α and thus probably the material from an emptying prominence; a 'dark' shell surrounding the loop, probably identifiable with the cavity seen around prominences in the low corona; and an outer 'rim' of displaced coronal material. Such ejections, representing a discrete addition of material to the solar wind, were detected on average 0.9 times per day during the 1980 Solar Maximum Mission sampling period — 20 per cent more often than detected by Skylab in the declining phase of the previous sunspot cycle in 1973–4. (From Hundhausen, A.J., Sawyer, C.B., House, L., Illing, R.M.E. and Wagner, W.J. *Journal of Geophysical Research* **89**, 2639; 1984.)

Tumour virology

Papilloma viruses and cervical tumours

from Lionel Crawford

THE epidemiology of cancer is, in general, not what would be expected for a group of diseases caused by infectious agents but there are some exceptions. Tumours of the uterine cervix and elsewhere in the female genital tract as well as cancer of the penis appear to involve infection. Cervical cancer is strongly correlated with sexual promiscuity both of women and their male partners^{1,2}. Genital warts, which are caused by papilloma viruses, are sexually transmitted and their incidence is also correlated with promiscuity. About half the women with vulvar genital warts examined in one study also had evidence of abnormalities of the cervix³. It was natural, then, for zur Hausen to ask whether papilloma viruses are implicated in the causation of cervical tumours as well as genital warts⁴. The answer seems likely to be yes.

The incidence of genital warts, the incidence of cervical cancer and the mortality from cervical cancer, especially among women under 35 years old, have all increased markedly over the last two decades^{5,6}. If we are to believe the results of surveys which have featured so prominently in the Sunday papers, there has been a substantial increase in sexual activity in this country over the same period, coupled with a decrease in the age of first sexual experience. The increasing mortality from cervical cancer is particularly striking since, together with lung cancer (associated with smoking cigarettes and still rising in women), the overall mortality due to cancer has been falling slowly. This adds urgency to the efforts being made to identify an infectious cause of cervical tumours. Intrinsically, the problem is difficult because any sexually transmitted agent is likely to be more frequently present in promiscuous than celibate or non-promiscuous women, so a simple demonstration of the presence of virus or its traces in tumours is by no means enough. Certainly most women with cancer of the cervix have been exposed to papilloma virus; one study reports antibodies against a papilloma virus group specific antigen in the serum of 93 per cent of cervical cancer patients⁷, and, surprisingly, none in the controls.

It would be much more convincing if evidence of persistent viral DNA could be found in tumours (although this is not required if the mechanism of transformation is postulated to be 'hit and run', as has been suggested for herpes viruses). Repeated efforts to show that herpes simplex type II DNA persists in cervical tumours have produced some positive results

but at best in only a small minority of tumours. Similar studies with papilloma viruses were initially also rather disappointing but the reasons are now clear. Human papilloma viruses (HPVs) comprise a very heterogeneous group with more than 24 different types already isolated. The different types are generally only weakly related by the criterion of nucleic acid hybridization. Thus, in general, it is necessary to probe tumour tissue with the corresponding HPV DNA probe if the presence of a particular type of HPV is to be reliably detected.

Largely as a result of the efforts of zur Hausen and his group in Germany, DNAs from several previously unknown types of HPV have recently been cloned and characterized. Four of the types — HPV 6, 11, 16 and 18 — are commonly associated with various lesions of the genital tract. Types 6 and 11 are associated with genital warts (condylomata acuminata) and with premalignant lesions of the cervix but rarely with carcinomas^{8,9}. Conversely, types 16 and 18 are predominantly associated with tumours. In one study, HPV 16 DNA was detected in over 60 per cent of the cervical tumours examined but less often in condylomata acuminata where it was found in conjunction with the more usual HPV 6 and 11 (ref. 10). In premalignant lesions of the cervix, HPV 16 is most frequently found in association with histologically abnormal mitotic figures, suggesting that the lesions are likely to progress to malignancy¹¹; the same may be true for lesions elsewhere in the genital tract¹². Seven out of eight carcinomas *in situ* of the vulva were recently found to contain HPV 16 (D. McCance, personal communication). HPV 18 is also found predominantly in tumours; zur Hausen's group report its presence in about 25 per cent of cervical tumours and in three out of four cervical tumour cell lines (including HeLa and KB lines which have been passed extensively in culture), but its absence from benign tumours and premalignant lesions¹³.

Clearly the situation is complex but it all adds up to a strong association of HPV with cervical tumours, particularly HPV 16 and 18. The other types, 6 and 11, may be associated with premalignant lesions and could also act as helper viruses for HPV 16 and 18. As with other tumours, there may be several stages between normal cervical cells and the fully malignant carcinoma cells, with different agents involved at different stages. These could be different HPV types or other viruses such as herpes simplex type II, or non-viral agents. Work with papilloma viruses in humans and

animals suggests that ultraviolet or ionizing radiation and chemical carcinogens or promoting agents act in concert with papilloma viruses to produce malignant tumours. Everyone working with HPV and cervical tumours has been careful to emphasize that what has been shown is an association between HPV and cervical tumours with no proof that they actually cause the tumours. The expectation would be that, if they did so, it would be in conjunction with other agents.

The details of HPV structure and replication are in the process of being unravelled and it is already clear that they are very different from simian virus 40 and polyoma virus in their genome size, organization and expression. They are also probably quite different in their mode of transformation and analogies with the smaller papova viruses are likely to be misleading rather than useful. In condylomata acuminata and in premalignant lesions, papilloma virus usually exists in the form of free episomal DNA, whereas HPV 16 and 18 may be integrated into chromosomal DNA in carcinomas (ref. 13 and D. McCance, personal communication). It is not clear what part rearrangement of the viral DNA or integration may play in transformation and whether integration is into specific sites in the cellular DNA. None of these uncertainties detracts from the importance of the now very impressive body of evidence implicating papilloma viruses with cervical and also with penile cancer. If they play an essential part in one or more stages of the malignant process the best prospect yet for intervention and control of these tumours will be on offer. □

I am indebted to Dr D. McCance, Guys Hospital, for his help and advice and for permission to quote his unpublished results.

1. Buckley, J.D. *et al.* *Lancet* ii, 1010 (1981).
2. Singer, A. *J. Rep. Med.* **28**, 109 (1983).
3. Walker, P.G. *et al.* *Br. J. vener. Dis.* **59**, 327 (1983).
4. zur Hausen, H. *Cancer Res.* **36**, 794 (1976).
5. Roberts, A. *Health Trends* **14**, 41 (1982).
6. Oriel, J.D. *Dermatologic Clinics* **1**, 93 (1983).
7. Baird, P.J. *Lancet* ii, 17 (1983).
8. Gissmann, L. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 560 (1983).
9. McCance, D.J. *et al.* *Br. med. J.* **287**, 784 (1983).
10. Durst, M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 3812 (1983).
11. Crum, C.P. *et al.* *New Engl. J. Med.* **310**, 880 (1984).
12. Ikenberg, H. *et al.* *Int. J. Cancer* **32**, 563 (1983).
13. Boshart, M. *et al.* *EMBO J.* **3**, 1151 (1984).

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In the article 'Progress in malignancy' by Miranda Robertson (*Nature* **309**, 512; 1984), the last sentence of the first paragraph should have read 'At the same time, Schwab *et al.*, who originally discovered the *N-myc* gene², report that in neuroblastomas *N-myc* amplification is associated with late stages of tumour progression, and thus may have prognostic implications'.

Molecular endocrinology

Vasopressin, tissue-specific defects and the Brattleboro rat

from T.I. Bonner and M.J. Brownstein

WITHOUT the Brattleboro rat, we would be short of a good animal model in which to study the importance of the mammalian antidiuretic hormone, vasopressin. Brattleboro rats inherit diabetes insipidus as a recessive trait; homozygous animals excrete large volumes of dilute urine and suffer from extreme thirst, and they lack the normal content of vasopressin in their posterior pituitary. Had the genetic defect of the Brattleboro rat turned out to be a simple deletion of the vasopressin gene, matters would be simple but uninteresting. Several recent papers reveal a much more intriguing situation.

Vasopressin is synthesized in the cell bodies of neurones of the hypothalamic region of the brain in the form of a precursor which also contains two other peptides, neurophysin and a nameless glycopeptide. The precursor is packaged into vesicles and is enzymatically processed into its constituent peptides during the passage of the vesicles, within the axons of the neurones, to the posterior pituitary. The vesicular contents are released into blood vessels in the pituitary to act on peripheral target tissues. If the posterior pituitaries of normal animals are removed, they develop diabetes insipidus like the homozygous Brattleboro rat.

In attempting to define the genetic defect of the Brattleboro rat, Schmale and Richter have recently cloned a vasopressin gene from one such animal and have shown that it has a single-nucleotide deletion within the second of three exons¹. We should note at the outset that it is a formal possibility that this deletion is not the controlling defect since it has neither been shown that the rat is homozygous for the deletion nor that it is present in more than one Brattleboro rat. Taken at face value, however, the deletion results in a frameshift which alters the amino acid sequence of one-third of the neurophysin, eliminates the arginine by which the glycopeptide is cleaved from it, completely alters the sequence of what would have been the normal glycopeptide and removes its glycosylation site, the normal termination codon. By removing 5 of the 13 cysteine residues normally present in the neurophysin, the frameshift results in a precursor that may be so drastically altered that it is no longer properly processed or transported. Schmale and Richter also speculate that the absence of a termination codon could impede translation of the mRNA by preventing ribosomes from disengaging from mRNA, a proposal which should be tested by deleting the termination codon of the normal gene and

assaying its translation efficiency. The gene defect of the Brattleboro rat could account for the presence of small amounts of 'peptide X' in Brattleboro hypothalami². This peptide is similar in size to the normal vasopressin precursor and has vasopressin- and neurophysin-like immunoreactivity, but is not glycosylated.

Although Schmale and Richter find equivalent amounts of vasopressin mRNA in Brattleboro and normal rats, suggesting that there is no defect in transcription, this result is disputed by Majzoub *et al.* who find that dehydrated Brattleboro rats have only 5 per cent of the hypothalamic vasopressin mRNA of similarly dehydrated Long Evans rats³. Since dehydration of the Long Evans rat results in a 20-fold increase in hypothalamic vasopressin mRNA, their conclusion is that the Brattleboro defect is transcriptional rather than translational.

Recently vasopressin has been detected in ovaries, adrenals (see references cited in refs 4 and 5) and sympathetic ganglia⁶ of normal rats. One might have predicted that the Brattleboro rat would have no vasopressin in its adrenal or ovary, just as it has none in its pituitary, but this seems not to be the case. On page 64 of this issue of *Nature*, Nussey and co-workers report that the adrenals of homozygous Brattleboro rats have a normal content of vasopressin-like material as measured by radioimmunoassay, and that the material has the same chromatographic properties as authentic vasopressin, though one might quibble over the chromatographic resolution⁴. Moreover, it is biologically active and its antidiuretic action is blocked by a specific vasopressin antagonist. Similarly, on page 61, Lim *et al.* show that the ovaries of Brattleboro rats contain as much vasopressin-like peptide as ovaries from Long Evans rats⁵. In the ovary, the peptide is confined to luteal cells; in the adrenal, it is present in chromaffin cells. Despite the fact that neither the adrenal nor the ovary vasopressin-like peptide has been purified and chemically characterized, it seems likely that they are both the same nonapeptide as is present in the posterior pituitary of normal animals. Thus, the defect in vasopressin production in the Brattleboro pituitary seems to be tissue specific; how the tissue-specificity can be reconciled with the gene defect is unclear.

One possible explanation is that there is a second gene for vasopressin or a closely related peptide and it is this gene that is expressed in the adrenal and ovary. There is a precedent for this suggestion in marsupials, which produce two

vasopressin-like peptides⁷. The only datum relevant to the rat is a Southern blot of rat DNA using the whole rat vasopressin gene as probe¹ but at such high stringency that even the closely related oxytocin gene was not detected. Thus, this experiment only excludes the presence of a second gene if it is nearly identical to the first.

A second possibility is that there is tissue-specific splicing of the vasopressin RNA which results in the substitution of an alternate exon for the defective second exon in the adrenal and the ovary. Both hypotheses predict that the size of the vasopressin mRNA in the adrenal and ovary would be different from that in the hypothalamus; characterization of these mRNAs will be critical.

It would be helpful to know whether immunoreactive neurophysin and glycoprotein are present in Brattleboro adrenals and ovaries. The observation of an immunoreactive glycopeptide in Brattleboro adrenals but not pituitary (E. Mezey, personal communication) is consistent both with the existence of a second gene that encodes a similar glycopeptide (the oxytocin gene does not) and with alternate splicing which retains the third exon encoding the glycopeptide. Examination of the rat and bovine gene sequences^{1,8} indicates that an alternate second exon may be present in the large intron between the first and second exons (nucleotides 892–952 of the bovine sequence).

Despite preliminary evidence (the presence of the glycopeptide in Brattleboro adrenal) to the contrary, the vasopressin message in the adrenal and ovary of the Brattleboro rat may prove to be the same abnormal message that is found in the hypothalamus. If so, it would imply that the abnormal precursor gives rise to normal levels of vasopressin in the adrenal and ovary but not in the pituitary because of a tissue-specific post-translational defect. The most trivial explanation would be that Brattleboro hypothalamic neurones make vasopressin but it is released as rapidly as it is formed. Alternatively, cells in the adrenal and ovary may handle the abnormal precursor differently than neurones do. It may be possible to explore these possibilities with careful pulse-labelling studies, but it seems that until we understand the roles of the various components of the vasopressin precursor, we shall not understand the Brattleboro rat's deficit. □

- Schmale, H. & Richter, D. *Nature* **308**, 705 (1984).
- Russell, J.T., Brownstein, M.J. & Gainer, H. *Endocrinology* **107**, 1880 (1980).
- Majzoub, J., Pappey, A., Burg, R. & Habener, J. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Nussey, S.S. *et al.* *Nature* **310**, 64 (1984).
- Lim, A.T.W. *et al.* *Nature* **310**, 61 (1984).
- Hanley, M.R. *et al.* *Nature* **309**, 258 (1984).
- Chauvet, M.T., Hurpet, D., Chauvet, J. & Acher, R. *Gen. comp. Endocr.* **49**, 63 (1983).
- Ruppert, S., Scherer, G. & Schutz, G. *Nature* **308**, 554 (1984).

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Homocysteine and arteriosclerosis

SIR — Spurred on by a recent book¹, I would like to remind *Nature's* readers that the cholesterol theory is not the only one that could explain arteriosclerosis; the homocysteine theory has been developing since the 1960s as the result of studying youngsters with a disease known as homocystinuria. Victims of homocystinuria usually die of vascular complications between the ages of 7 and 13, but in some cases the high homocysteine levels are responsive to vitamin B₆ therapy and some people with homocystinuria are now saved.

An insufficient amount of vitamin B₆ will result in contact inhibition causing smooth cells to grow abnormally^{2,3}. The connection lies in the consumption of the amino acid methionine, which is ultimately metabolized to homocysteine. Vitamin B₆ is required for the further metabolism of homocysteine, and if this transformation is inhibited, homocysteine can build up. Homocysteine causes smooth cells to grow abnormally. Since areas of abnormal smooth cell growth in arterial endothelial lining may become sites of cholesterol plaque formation, cholesterol and other lipid accumulation may then be a direct consequence of the abnormal smooth cell growth. Many people generally consider that there is (or must be) enough vitamin B₆ in their diet. However, in 1955 Henry Schroeder found that in the United States the foods in the typical diet have low levels of vitamin B₆ due to processing and cooking, and that the diet contains an insufficient amount of B₆ during most of the year⁴.

As eloquently presented in a review article by H.C. McGill Jr, there exists no correlation between dietary cholesterol and serum levels of cholesterol⁵. Interestingly, serum cholesterol can be modulated by sucrose (table sugar). In 1964 Winitz *et al.* reported that as sucrose was increased from 0 to 25% of the total carbohydrate source in a defined diet of normal adults, their serum cholesterol levels increased concomitantly from 160 to 208 mg per cent. Subsequently, when the diets were changed to 100% glucose carbohydrate source (sucrose free) for an eighteen-week period, the average serum cholesterol level fell to 151 mg per cent^{6,7}.

This raises three questions. (1) Would it be appropriate to focus more attention on the alternate theory of arteriosclerosis, namely, the homocysteine theory (the lack of vitamin B₆)? (2) Why is sucrose never discussed or alluded to as a potential problem associated with raised cholesterol levels (sucrose being one of the few commodities that we ingest in large amounts daily)? (3) Very few of my colleagues or friends take vitamins; in this society where a lot of our food is cooked fast, well done, and with lots of protein, should we be more concerned with taking some vitamins daily? We must make sure that the role essential

vitamins play in our health is not overlooked because of recent controversy over megavitamin therapies.

There is a danger that this most interesting theory (the homocysteine theory) and the sucrose involvement may be overlooked. If these concepts are as important to every one of us as they seem, it would be a shame for them to go unnoticed one minute longer.

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1. Gruberg, E.R. & Raymond, S.A. *Beyond Cholesterol, Vitamin B₆, Arteriosclerosis, and Your Heart* (St Martin's Press, New York, 1981).
2. McCully, K.S. *Am. J. Path.* **56**, 111 (1969); **59**, 181 (1970); **61**, 1 (1970); *Am. Heart J.* **83**, 571 (1972).
3. Levene, C.I. & Murray, J.C. *Lancet* **i**, 628 (1977).
4. Schroeder, H.A. *J. chron. Dis.* **2**, 28 (1955).
5. McGill, H.C. Jr *Am. J. clin. Nutr.* **32**, 2664 (1979).
6. Winitz, M., Graff, J. & Seedman, D.A. *Archs Biochem. Biophys.* **108**, 576 (1964).
7. Winitz, M., Seedman, D.A. & Graff, J. *Am. J. clin. Nutr.* **23**, 525 (1970).

Fungi in culture

SIR — During work in connection with the UK National Collection of Fungus Cultures, it has emerged that too many authors of papers which use fungus cultures do not (1) have the identity of the material checked by a specialist, and/or (2) deposit a sub-culture in a national collection where it can be preserved and so made available to later workers (or even the same worker if his cultures die or become contaminated). Too much elegant biochemical and chemical work in genera such as *Penicillium* and *Trichoderma* is already unusable because of uncertainties over the identity of material studied for which isolates were not preserved in a public collection. The UK National Collection of Fungus Cultures, held at this institute since 1947, already holds about 11,000 strains in pure culture, by a wide variety of methods (depending on the fungi concerned). [See *The Preservation and Maintenance of Living Fungi*, Commonwealth Mycological Institute, £6.50 post free.]

Isolates to be cited in publications are normally accepted for deposit free of charge, and can be preserved and held in confidence in advance of publication. The institute is also an international depository for patent strains and operates a safe-deposit service.

Where fungi have not been checked by an expert, the institute staff of about 70 includes 16 specialist taxonomists who provide a world identification service. This service is free for non-commercial UK submissions.

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Oil-shale petroporphyrin

SIR — I was interested to learn that Ekstrom *et al.* determined the X-ray structure of the naturally occurring petroporphyrin, vanadyl deoxophyllerythroetioporphyrin (VO-DPEP) (*Nature* **306**, 173; 1983). The authors did not mention, but I would like to add, that the X-ray structure of synthetic VO-DPEP was published in 1968 and 1969^{1,2}. From the structure diagrams of Ekstrom, the synthetic and natural VO-DPEP are identical. The crystal structures are not identical because 1,2-dichloroethane is present as solvent of crystallization in synthetic VO-DPEP. Ekstrom's unit cell packing diagram looks as if that structure contains chloroform as a solvent of crystallization. This is logical because the unit cell volumes are very similar (synthetic: 3,149 Å³; natural: 3,167 Å³).

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1. Pettersen, R.C. & Alexander, L.E. *J. Am. chem. Soc.* **90**, 3873-5 (1968).
2. Pettersen, R.C. *Acta crystallogr.* **B25**, 2527-39 (1969).

Canary before the pigeon

SIR — Epstein *et al.*¹ recently reported the replication of W. Köhler's one-box problem but with the bird (pigeon) as the subject instead of the chimpanzee, as was the case in Köhler's experiments. But Epstein *et al.* overlooked the relevant literature with the bird as the subject. About thirty years ago, I undertook the replication of Köhler's one-box problem as well as the replication of Köhler's stacking problem (that is, stacking two, three or four boxes) with the bird as the subject (canary). Of course, Köhler's procedure was appropriately modified to suit the bird's anatomy. I reported the canary's achievements in several journals²⁻⁴, one of which contains photographs⁴. Photographs also were published in textbooks⁵ and in the popular media⁶.

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1. Epstein, R., Kirshnit, R.P., Lanza, R.P. & Rubin, L.C. *Nature* **308**, 61-62 (1984).
2. Pastore, N. *J. comp. physiol. Psychol.* **47**, 288-289 (1954).
3. Pastore, N. *Psychol. Rep.* **1**, 307-315 (1955).
4. Pastore, N. *Scient. Am.* **192**, 72-79 (1955).
5. Welty, J.C. *The Life of Birds*, 173 (Saunders, Philadelphia 1962).
6. *MD*, 100-101 (March, 1960).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

Rotational frequency splitting of solar oscillations

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Prograde and retrograde sectoral oscillations of the Sun have been observed so as to determine frequency differences produced by rotation. Oscillations in the frequency range 2.1–3.7 mHz and with spherical harmonic degrees from 1 to 100 have been identified. Average frequency shifts due to rotation in a sidereal reference frame are found to range from a high of ~660 nHz at degree 1 to a low of ~423 nHz at degree 6, rising to ~471 nHz at degree 100. These results indicate that most of the Sun's volume rotates at a rate close to that of the surface, but also that the energy-generating core may rotate more rapidly than the surface.

SINCE the development of the use of solar oscillations as a means of probing the Sun's interior, there has been much interest in determining the internal rotation. An accurate determination would help us to address questions of the evolution and internal structure of the Sun, the solar gravitational quadrupole moment, the transfer of angular momentum in the convection zone and the properties of the dynamo that is presumed to produce solar activity.

The observational approach depends on two characteristics of solar acoustic (p-mode) oscillations. First, oscillation modes of different spherical harmonic degree, l , and radial order, n are trapped in cavities that span different depth ranges¹. Modes of different angular order, m , also extend over different latitude zones. Second, the oscillation frequencies of a mode, ν_{nlm} , are shifted by rotation^{2–6}. The amount of the shift depends on the structure of the interior, the rotation frequency and its variation with radius and latitude. The dependence on internal structure can be calculated from a solar model. Consequently, measurements of the frequency shifts of a sufficiently large range of oscillation modes allow the variation of internal rotation with depth and latitude to be determined.

The first attempt to determine internal rotation in this way used high-degree acoustic oscillations that probe the outer few per cent of the equatorial zone of the Sun⁷. Frequency shifts were observed which implied that the rotation rate increases with depth; similar observations from Kitt Peak failed to confirm a deviation from solid body rotation⁸. Further observations suggested the importance of convective velocities on frequency shifts of high-degree modes⁹.

Claverie *et al.*¹⁰ found evidence of frequency splitting of low-degree acoustic oscillations that probe nearly the entire solar radius. The magnitude of the observed splitting seemed to imply that the core of the Sun rotates substantially faster than the surface. However, more frequency components were observed than were expected from the geometry of the observation, suggesting that the Sun contained an oblique magnetic rotator¹¹. This suggestion was not universally accepted⁶. Other low-degree observations¹² have not yet confirmed the rotational splitting observed by Claverie *et al.*

Hill *et al.*¹³ reported rotational splitting of low-frequency acoustic and gravity modes of degrees 4–10. They concluded that the Sun rotates about a single axis and that the core rotates ~6 times faster than the surface. Gough reached a similar conclusion using preliminary values from the same observations¹⁴.

Observations

We have undertaken a series of observations of the frequencies of oscillation modes with l ranging from 1 to 200 and with

$m = \pm l$ (sectoral harmonics). Our intention was to measure the rotational frequency splittings

$$\nu'_{nl} \equiv \frac{\nu_{n,l,m=-l} - \nu_{n,l,m=+l}}{2l} \quad (1)$$

over a sufficient range of l to allow the rotation variation over most of the solar radius to be determined. If the Sun rotated rigidly, ν'_{nl} would be the rotation frequency. We selected sectoral modes for an initial study because they exhibit the largest rotational frequency shifts. However, this restriction prevents us from separately resolving the radial and latitudinal variations of rotation. Observations were made at Kitt Peak every day throughout 10–26 May 1983 for an average of ~11 h per day. The observational technique was nearly the same as we described previously¹. Briefly, we formed an image of the entire disk of the Sun on the entrance slit of a large grating spectrograph. The slit was orientated perpendicular to the Carrington rotation axis and the image was optically averaged perpendicular to the slit. Two photospheric spectrum lines were recorded simultaneously by a two-dimensional diode-array camera every minute for up to 12 h each day. These data were used to produce a time series of the Doppler shift of ~200 spatial elements from the east to west limbs of the Sun. The values of the Doppler shifts at each minute were multiplied by spatial functions (foreshortened sectoral harmonics, weighted by limb darkening and averaged perpendicular to the equator) that represented the signal we would expect to observe with our geometry arising from individual sectoral modes of degrees from 0 to 200. These functions are complex with the real part being even about disk centre and the imaginary part odd. The temporal power spectrum of the sequence of projections for a given degree then has the prograde ($m = -l$) and retrograde ($m = +l$) sectoral modes separated cleanly into positive and negative frequency components. This reduction technique is similar to the spatial-temporal analysis used to study high-degree modes⁷ except that the spatial part of the transform is modified. The clean separation of prograde and retrograde modes in the present data allows us to measure the rotational splitting even when the spectral features are broad. At low degree, this is the main advantage that the present, spatially-resolved observations have over previous attempts to measure rotational splitting using unresolved observations.

Night-time gaps in the time series were set to zero velocity before the temporal transform was computed. Thus, the resulting power spectra have a frequency resolution of ~700 nHz and strong frequency sidelobes at spacings of $1 \text{ day}^{-1} = 11.6 \mu\text{Hz}$. The latter feature is particularly annoying for later analysis. We have experimented with various methods to eliminate the

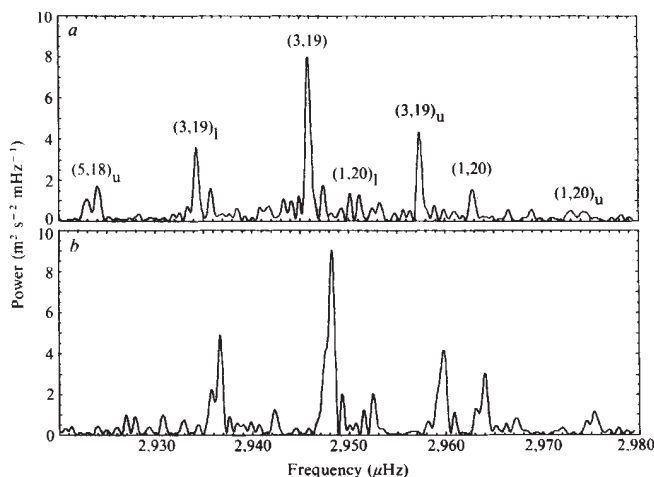


Fig. 1 Portions of the degree 3 power spectra of retrograde (a) and prograde (b) modes. The various features are labelled (l, n) with subscripts u and l to denote upper and lower frequency sidelobes of main features. Leakage of degree 1 and 5 modes into these spectra is obvious.

sidelobes and find that a modification of the CLEAN algorithm described in ref. 15 works well. Some of the present results are derived from 'cleaned' spectra.

The response of our observation and reduction method to modes associated with spherical harmonics other than those intended is a complicated function of l and m . Roughly speaking, a given spatial analysis responds to a range of about five consecutive values of l centred on the one of interest. In our analysis this leakage sometimes causes confusion between modes of various degrees. There is also a response to non-sectoral modes that is discussed later.

Analysis and results

Our first analysis of frequency shifts caused by rotation was to cross-correlate the prograde and retrograde sectoral spectra in the frequency range from 2–4 mHz. This analysis immediately implied that ν'_{nl} for degrees 1–200 is not very different from the surface rotation frequency. However, this analysis was contaminated by the leakage of unwanted modes into a given cross-correlation. To avoid this problem, we constructed masks that passed only the desired modes and again tried cross-correlations of the prograde and retrograde spectra—this procedure was only moderately successful.

The present results are based on measurement and analysis of the frequencies of individual modes, and will be refined in future work. Some examples of low-degree modes are shown in Fig. 1. Mode frequencies were determined by several methods. One method was to position the cursor of an interactive graphics terminal on what appeared to be the centroid of a selected mode on a plot of the power spectrum and to record the frequency of the cursor position. This method was used for $l > 10$. Another method, used for $l < 20$, was to fit a Voigt line profile by least-squares to the observed spectral features and to record the central frequency of the fitted profile. An additional method used the CLEAN algorithm¹⁵. A portion of the spectrum was 'cleaned' with a gain factor of 0.1 until the residual spectrum appeared to be just random noise. The first moment of the power distribution in the clean spectrum over a frequency bandwidth of 8 μ Hz centred on the feature yielded the frequency estimate. This method was applied to spectra with $l < 5$. When discrepant results from more than one method were found in excess of a few tenths of 1 μ Hz we used moment and Voigt profile techniques in order of preference. The frequencies of the prograde and retrograde modes of a given degree and radial order were combined using equation (1) to yield the rotational splittings ν'_{nl} . These splittings were converted from a synodic to a sidereal

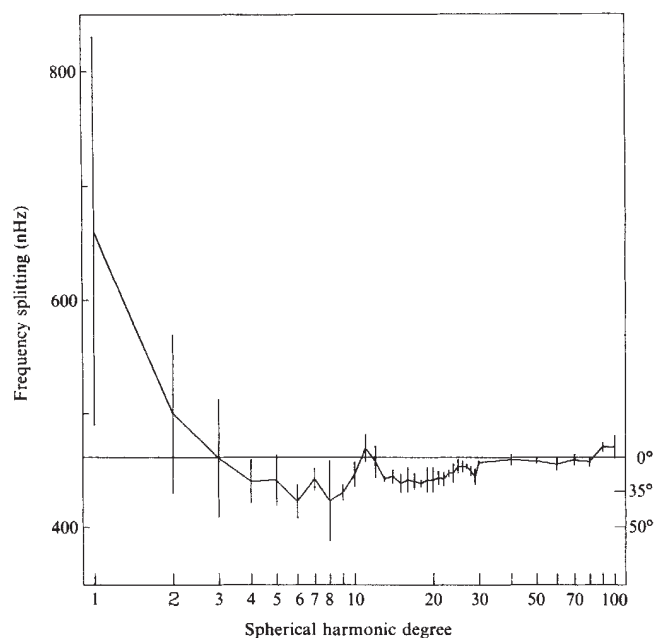


Fig. 2 The mean shift of solar p-mode oscillations due to rotation, ν' , is shown for a range of spherical harmonic degree. The measurements have been corrected for sensitivity to non-sectoral modes. The error bars represent the standard deviation of the mean. There is substantial uncertainty at low and high degree. The frequency reference frame is sidereal and the scale of surface rotation frequency versus latitude at the right is based on the motion of magnetic features¹⁸. The horizontal line is the surface equatorial rotation frequency of 462 nHz.

reference frame by adding 31 nHz and then averaged with equal weight for a given value of degree to produce the values ν'_i presented in Table 1 and plotted in Fig. 2.

The quoted standard deviation of the mean is the standard deviation of the population of values divided by the square root of the size of the population. This is perhaps an optimistically small measure of random error because we average only a few numbers at a time. For degrees 1 and 2, the formal errors were smaller than we felt were reasonable so we estimated that the error of each frequency measurement was 340 nHz. This value was determined by the scatter of degree 4, 5, 9 and 10 measurements, of which we had a relatively large number. Based on the appearance of the single $l = 100$ mode, the frequency error was estimated to be 2.5 times the error for degree 90. The random errors of our frequency measurements are caused by background noise and spectral fine structure. Noise is important at low frequencies but the major source of trouble is spectral fine structure. Although cases of a single, narrow spectral peak corresponding to a particular oscillation are seen, we typically observe multiple peaks within a more or less broad envelope.

There are at least three possible causes of the spectral fine structure. First, our observational suppression of near-sectoral modes is not perfect so that several closely-spaced modes may appear where ideally we would expect only the sectoral mode. The result is that beating of these almost-resolved modes may produce spectral fine structure that would be absent in observations with better resolution. Second, the sectoral and near-sectoral modes of a given multiplet may be excited to different amplitudes. This effect could produce noisy fine structure within a broad envelope and it also explains the occasional appearance of single, narrow peaks. The third factor is the possibility that the modes are coherent for times shorter than the duration of our observations. This effect is required to explain the frequent cases of envelopes wider than can be explained by the first two causes. This indicates that many oscillation modes are short-lived, as found previously^{12,16}. To investigate the lifetime question further we selected some low-degree modes for which our

Table 1 Average sidereal frequency splittings

Degree (<i>l</i>)	Observed splitting, ν'_l (nHz)	Corrected splitting (nHz)	Frequencies of averaged modes, $\nu_{n,l}$ (mHz)	Degree (<i>l</i>)	Observed splitting, ν'_l (nHz)	Corrected splitting (nHz)	Frequencies of averaged modes, $\nu_{n,l}$ (mHz)
1	660 ± 170	660	2.56, 2.96	18	432	439	2.72, 2.87, 3.30
2	500 70	500	2.08, 2.76, 2.89	19	434	442	2.47, 2.90, 3.19, 3.33
3	460 52	461	2.81, 2.95, 3.08, 3.35	20	434	442	2.20, 2.50, 2.64, 2.79, 3.08, 3.22, 3.37
4	440 19	441	2.32, 2.46, 2.59, 2.73, 2.86, 3.13, 3.27	21	436	444	2.23, 2.53, 2.68, 2.82, 3.11, 3.26, 3.55
5	440 22	442	2.10, 2.24, 2.37, 2.51, 2.64, 2.78, 3.46	22	435	443	2.26, 2.41, 2.56, 2.71, 2.85, 3.00, 3.15, 3.29, 3.44, 3.58
6	420 15	423	2.28, 2.69, 2.82, 2.96	23	440	448	2.13, 2.74, 2.89, 3.62
7	440 10	443	2.32, 2.59, 3.01, 3.28	24	440	448	2.32, 2.47, 2.92, 3.07
8	420 35	424	2.22, 2.50, 2.77, 3.32	25	446	454	2.18, 2.34, 2.49, 2.65, 2.80, 2.95, 3.24
9	426 7	431	2.13, 2.26, 2.40, 2.68, 2.95, 3.09, 3.51	26	447	454	2.37, 2.52, 2.83, 2.98, 3.13, 3.57
10	442 11	447	2.16, 2.30, 2.44, 2.58, 2.86, 3.27, 3.69	27	446	454	2.23, 2.55, 2.70, 2.86
11	464 12	470	2.48, 2.62, 2.90, 3.17	28	442	450	2.25, 2.42, 2.57, 3.19, 3.34
12	452 14	458	2.38, 2.52, 3.08, 3.35	29	438	445	2.76, 2.91, 3.07
13	437 2	443	2.41, 2.69, 2.83, 2.97, 3.11, 3.25, 3.68	30	450	457	2.78, 2.94, 3.09
14	438 6	445	2.45, 2.59, 2.73, 2.87, 3.15, 3.43	40	453	460	2.50, 2.68, 3.02, 3.19, 3.35
15	432 8	439	2.47, 2.62, 2.76, 2.90, 3.04, 3.18	50	452	459	2.86, 3.05, 3.23
16	435 11	442	2.37, 2.66, 2.80, 2.94, 3.08, 3.22	60	449	456	2.44, 2.85, 3.23, 3.41
17	434 7	441	2.26, 2.40, 2.55, 2.69, 2.98, 3.12, 3.26	70	453	460	2.36, 2.79, 3.00, 3.20
				80	451	458	2.23, 2.47, 2.70, 2.92, 3.35
				90	464	471	2.33, 3.05
				100	464	471	2.42

ability to isolate individual modes is good. The time variation of amplitude and frequency of the individual modes was investigated by computing their analytic signal¹⁷ representation. We find characteristic times of amplitude and frequency fluctuations of typically a few days (but with a large range). This suggests a genuinely short lifetime for some modes but much more work is needed. Whatever the cause of spectral fine structure, the width of the spectrum of typical oscillation modes is several times larger than our frequency resolution of 700 nHz, and it is hard to measure an accurate average frequency. This problem is most severe at high frequency and high degree. For these reasons, only about one-third of the modes we examined are included in Table 1; the other modes are too ill-defined.

Apart from random noise effects in our results, there are also systematic effects. Our observational geometry presumes that the Carrington rotation axis is the correct one. If the true rotation axis is different, the observed amplitude of the sectoral modes would be reduced and we might incorrectly identify a spectral feature as sectoral when it was not. Such a misidentification would lead to a smaller rotational splitting. We believe that the error of aligning our observations with the Carrington axis is only of the order of 0.1 degree. This would produce a negligible change in the amplitudes of sectoral harmonics. The remaining issue is whether the Carrington axis applies to the entire Sun. The present results appear to be consistent with a single rotation axis but we cannot exclude other possibilities without further analysis.

The sensitivity of our observations to near-sectoral modes discussed earlier produces an important systematic effect. This is significant at larger degrees because our observing geometry does not then distinguish well between sectoral and near-sectoral modes of the same degree. Such modes are shifted by a few μ Hz less than the sectoral modes and are not well resolved in our spectra. This could lead to a systematic underestimate of the sectoral mode shifts. We calculated the size of the effect by assuming that sectoral and near-sectoral modes are excited to equal amplitudes. The corrections do not exceed 8 nHz and are included in the results listed in Table 1 and plotted in Fig. 2.

Another possible source of systematic errors is mode misidentification. In some areas of the $l-\nu$ spectrum we are certain

that the modes are correctly identified while in other areas we are less confident. The difficult areas are those in which $\Delta\nu = \nu_{n,l+1} - \nu_{n,l} < 20 \mu$ Hz. The difficulty increases as $\Delta\nu$ decreases below 20 μ Hz and is caused by a combination of temporal sidelobes, significant response to several degrees and non-sectoral modes and the short lifetime of modes. Therefore, we have been unable to perform the mode identification process¹ for $l > 100$.

Conclusions

Bearing in mind the observational limitations, what are the significant results? Starting at high degree, Fig. 2 shows a rotational splitting value slightly larger than the equatorial rotation frequency of surface magnetic features¹⁸. Proceeding to lower degrees we find a smaller rotational splitting. The reason for the increase at degrees 10–12 is not clear. An increase in rotational splitting starts at degree 3 and reaches a maximum at degree 1. The low-degree increase is not well determined and we admit the possibility of either no low-degree increase or a substantially larger one.

Deducing the internal solar rotation from these results is not simple because the observed rotational splittings are radial and latitudinal averages. The high-degree sectoral modes are strongly concentrated near the surface and the equator. For decreasing degree, both the depth and latitude penetration increase. If we suppose that there is little or no latitude variation of solar rotation we can form a rough idea of the radial variation by successively examining the splitting measurements that average over successively greater depths beneath the surface. Thus, the $l=90, 100$ results that pertain to shallow depths suggest that solar rotation is faster than the surface rate just below the surface. The lower degree results that average to greater depths indicate that the rotation rate gradually decreases inward to well below the convection zone until a rapidly-rotating region corresponding roughly to the energy-generating core is encountered. Extremely rapid rotation within the Sun, except possibly for the inner core, seems to be excluded by our measurements. When we compare this conclusion with the contribution of rotation at various radii to the net measure of the gravitational quadrupole moment of the Sun¹⁴, J_2 , we conclude that J_2 must be close to

the value computed for a rigidly rotating model of the Sun.

It is interesting to compare the present results with those of previous investigators referred to here as the Birmingham¹⁰, ACRIM^{12,19} and SCLERA¹³ groups. At degrees 1 and 2 our splitting results are consistent with the frequency width of the modes observed by the ACRIM group. Our measured splitting for $l = 1$ is within the upper limit set by the ACRIM data. The lifetimes of the modes that we deduce are also consistent with ACRIM results when one allows for the various definitions of lifetime that are used. Our degree 1 splitting result agrees well with the Birmingham result but the degree 2 results disagree. A possible explanation is that the short lifetimes of the modes plus mode beating effects produced frequency fine structure in the Birmingham data that was interpreted as being due to rotational splitting. This idea could also explain the appearance of more frequency components than expected in the Birmingham data. We did not observe the same modes as the SCLERA group but if one assumes that the value of rotational splitting is not a strong function of frequency for a given degree then our results do not agree. The SCLERA results are about three times larger than the splitting that we find for p-modes of the same degree;

the cause of this large discrepancy is not clear. Additional, independent frequency splitting measurements would be valuable.

Our measurements suggest that most of the Sun rotates slightly slower than the surface except for a more rapidly rotating core. The errors of measurement are such that we cannot exclude the possibility that the Sun rotates at a rate independent of radius, although this seems unlikely. A more complete interpretation of the measurements requires comparisons with model calculations—this is done in an accompanying article²⁰. Analysis of observations we made in collaboration with M. Pomerantz from the geographical South Pole is underway with the aim of distinguishing between latitude and radial rotation variations and to define the direction of the rotation axis with depth.

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1. Duvall, T. L. Jr & Harvey, J. W. *Nature* **302**, 24–27 (1983).
2. Cowling, T. G. & Newing, R. A. *Astrophys. J.* **109**, 149–158 (1949).
3. Ledoux, P. *Astrophys. J.* **114**, 373–384 (1951).
4. Hansen, C. J., Cox, J. P. & Van Horn, J. M. *Astrophys. J.* **217**, 151–159 (1977).
5. Gough, D. O. *Mon. Not. R. astr. Soc.* **196**, 731–745 (1981).
6. Gough, D. O. *Nature* **298**, 350–354 (1982).
7. Deubner, F. L., Ulrich, R. K. & Rhodes, E. J. Jr *Astr. Astrophys.* **72**, 177–185 (1979).
8. Rhodes, E. J. Jr, Harvey, J. W. & Duvall, T. L. Jr *Solar Phys.* **82**, 111 (1983).
9. Hill, F., Toomre, J. & November, L. J. *Sol. Phys.* **82**, 411–425 (1983).
10. Claverie, A., Isaak, G. R., McLeod, C. P. van der Raay, H. B. & Roca Cortes, T. *Nature* **293**, 443–445 (1981).
11. Isaak, G. R. *Nature* **296**, 130–131 (1982).
12. Woodard, M. & Hudson, H. *Nature* **305**, 589–593 (1983).
13. Hill, H. A., Bos, R. J. & Goode, P. R. *Phys. Rev. Lett.* **49**, 1794–1797 (1982).
14. Gough, D. O. *Nature* **298**, 334–339 (1982).
15. Cornwell, T. J. in *Synthesis Mapping* (eds Thompson, A. R. & D'Addario, L. R.) 9.1–9.14 (NRAO, Greenbank, 1982).
16. Grec, G., Fossat, E. & Pomerantz, M. A. *Sol. Phys.* **82**, 55–66 (1983).
17. Bracewell, R. N. *The Fourier Transform and Its Applications* 2nd edn (McGraw-Hill, New York, 1978).
18. Snodgrass, H. B. *Astrophys. J.* **270**, 288–299 (1983).
19. Woodard, M. F. & Hudson, H. S. *Bull. Am. astr. Soc.* **15**, 951–952 (1983).
20. Duvall, T. L. Jr *et al.* *Nature* **310**, 22–25 (1984).

Internal rotation of the Sun

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The frequency difference between prograde and retrograde sectoral solar oscillations is analysed to determine the rotation rate of the solar interior, assuming no latitudinal dependence. Much of the solar interior rotates slightly less rapidly than the surface, while the innermost part apparently rotates more rapidly. The resulting solar gravitational quadrupole moment is $J_2 = (1.7 \pm 0.4) \times 10^{-7}$ and provides a negligible contribution to current planetary tests of Einstein's theory of general relativity.

SOLAR oscillations can be used to probe the structure and dynamics of the Sun. In particular, rotation produces, in the spectrum of acoustic (p-mode) oscillations, fine structure that can be used to determine the internal rotation^{1–3}. Knowledge of the variation of rotation rate with depth and latitude would allow us to determine the gravitational quadrupole moment of the Sun, and would provide valuable information about the solar dynamo. Furthermore, we would gain new insight into the internal structure and history of the Sun.

The individual normal modes of oscillation of the Sun can be characterized by a radial order n , and a spherical harmonic degree l and azimuthal order m . In the absence of rotation and magnetic fields, each (n, l) set is $(2l+1)$ -fold degenerate with respect to m . Rotation breaks this degeneracy, producing rotational splitting in the frequency spectrum: modes propagating in the sense of the rotation are shifted to higher frequency, while modes propagating against the rotation are shifted to lower frequency. Acoustic oscillation modes of low degree penetrate

deeply into the interior, while high-degree modes are confined near the surface. Measurement of rotational splitting for modes of different degrees provides a means of determining the variation of rotation rate with depth.

Fine structure in the spectrum of low-degree solar oscillations, attributed to rotation, has been observed. Claverie *et al.*⁴ reported a fine structure consisting of $2l+1$ peaks in their spectra of observations of low-degree ($l=1, 2$) 5-min oscillations in integrated sunlight. If, however, the fine structure they saw were due to rotation about a single axis, their spectra should have shown only $l+1$ peaks. Isaak⁵ suggested that the fine structure was actually due to an inclined, rotating magnetic field much like that postulated earlier by Dicke⁶. Woodard and Hudson⁷ have established an upper limit of twice the surface rotational splitting for $l=1, 2$ modes. This limit is consistent with the result found by Claverie *et al.*⁴ Duvall and Harvey¹ reported a consistent value for $l=1$, but their value for $l=2$ differs from that of Claverie *et al.*⁴. The finite lifetimes of these modes has been

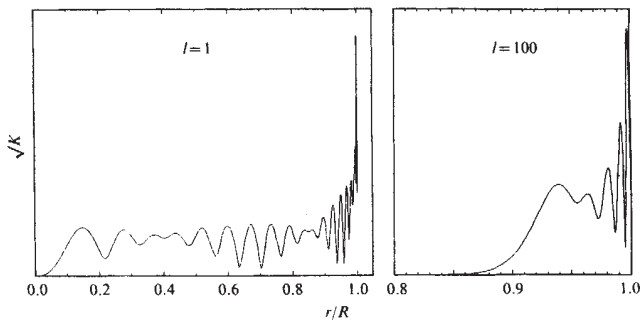


Fig. 1 The square roots of the averaged splitting kernels, $\sqrt{K_l}$, for $l=1$ and 100 as functions of fractional radius r/R . The $l=1$ kernel is an average of the two kernels corresponding with the two observed values of n . The ordinate scales are arbitrary.

suggested¹ as a possible source of the discrepancy.

Bos and Hill⁸ observed low-frequency oscillations of limb darkening. Hill *et al.*⁹ and Gough¹⁰ used these results to infer a steep gradient in the rotation rate in the solar convection zone and an interior rotating about six times more rapidly than the surface. On the other hand, low-frequency Doppler oscillations analysed by Delache and Scherrer¹¹ suggested that the region below the convection zone rotates approximately only half that rapidly.

Duvall and Harvey¹ present values for rotational splitting, about the surface axis, of prograde ($m=-l$) and retrograde ($m=+l$) sectoral oscillation modes in the frequency range 2.1–3.7 mHz and with l between 1 and 100. Frequency differences between 180 prograde and retrograde mode pairs were computed according to

$$\nu'_{nl} = \frac{\nu_{n,l,m=-l} - \nu_{n,l,m=+l}}{2l} \quad (1)$$

These were then averaged over observed values of n , with equal weight to increase significance without appreciably degrading the resolution in radius, for each l to produce 37 values of rotational splitting, ν'_l . We use these values here to determine the internal rotation rate of the Sun.

Rotational splitting

In general, the rotation rate may be a function of radius and latitude. However, we have little information on the dependence of the rate on latitude from sectoral modes alone. These modes are concentrated near the equator, between latitudes $\approx \pm l^{-1/2}$ when l is large, therefore, we really measure only an equatorially-concentrated average of the rotation rate over a varying, restricted range of latitude. Because of this concentration, sectoral modes are well suited for investigation of the radial variation of rotation near the equatorial plane. Assuming that the rotation frequency ν_{rot} depends only on radius r within an appropriate latitude range, the frequency splitting, ν'_{nl} , is given^{12,13} by

$$\nu'_{nl} = \int_0^R K_{nl}(r) \nu_{\text{rot}}(r) dr \quad (2)$$

where R is the solar radius, and K_{nl} is the splitting kernel, which we have calculated from two essentially equivalent solar models (ref. 14 and H. Saio, personal communication). The averaged rotational splitting ν'_l satisfies a similar equation with K_{nl} replaced by the uniformly weighted average of appropriate kernels, K_l . If ν'_l were known for a sufficient variety of oscillations, we could obtain $\nu_{\text{rot}}(r)$ by solving the averaged integral equation (2). We present the averaged splitting kernels, K , for the lowest ($l=1$) and highest ($l=100$) degree oscillations in Fig. 1 to illustrate the range of depth penetration for the acoustic modes that we used. Slightly more than half the contribution to the $l=1$ kernel comes from beneath the convection zone, whereas the $l=100$ kernel does not penetrate even to the base of the convection zone. Although the lowest-degree oscillations

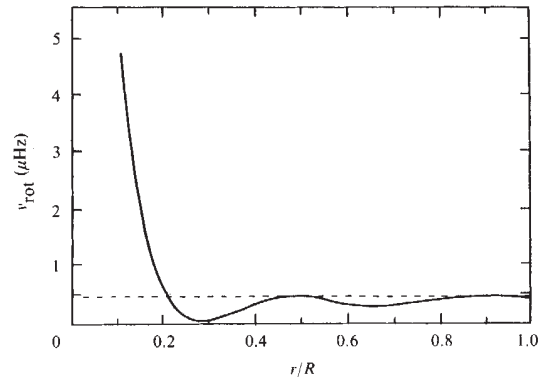


Fig. 2 An eighth-degree polynomial representation of rotation frequency $\nu_{\text{rot}}(r)$ as a function of the fractional radius r/R . For comparison, the surface rotation frequency at the equator is the dashed line at ~ 0.46 μHz .

do penetrate into the deep core, most of the information contained in these modes comes from the outer regions, and the calculated rotational frequency of the core must be the most uncertain.

Inversion of data

We present three different inversions of the integral equation (2): simple fitting of a polynomial, an optimal averaging procedure suggested by the work of Backus and Gilbert¹⁵, and a fitting of a piecewise constant representation of $\nu_{\text{rot}}(r)$. Figure 2 shows a least-squares fit of an eighth-degree polynomial representation of $\nu_{\text{rot}}(r)$ to the data. The solution is essentially flat for $r > 0.4R$, with only modest deviations from the equatorial surface rate. For $r < 0.4R$, where the results are less certain, the curve shows a dip and then a sharp rise with decreasing r . The general features of this fit are reproduced by the other two techniques. The curve is cut off for $r < 0.1R$, because only 1% of the deepest kernel ($l=1$) penetrates this region. All low-degree polynomial fits have structure similar to that shown in Fig. 2, although the positions of the maxima and minima vary with degree, and the rise at small values of r becomes more dramatic with increasing degree. Higher-degree polynomials show finer scale structure than the data can resolve.

The optimal averaging technique has the advantages that the depth resolution and error magnification are readily estimated and that a tradeoff can be made between these two quantities—increasing resolution leading to larger error magnification. The method seeks weighted averages, D , of the splitting kernels that are concentrated at different radii, r_0 and that have unit integral with respect to r :

$$D(r, r_0) \equiv \sum_l \alpha_{l,r_0} K_l(r) \quad (3)$$

If we were successful in concentrating D about r_0 , then D resembles the delta function, $\delta(r - r_0)$, and we might set

$$\nu_{\text{rot}}(r_0) \approx \int_0^R D(r, r_0) \nu_{\text{rot}}(r) dr = \sum_l \alpha_{l,r_0} \nu'_l \quad (4)$$

In our calculations, we have adjusted the parameter affecting the width of D so that the relative error in $\nu_{\text{rot}}(r)$ is small. The procedure was successful within the convection zone, $r > 0.7R$, with the results shown in Fig. 3. Unfortunately, the straightforward optimal averaging procedure fails to generate D 's that are well-concentrated deep in the Sun, because all of the K 's have large amplitudes near the surface. With only 37 different K 's there is no linear combination that is large in the core and that adequately cancels near the surface. To overcome this difficulty, we truncated the kernels by setting $K = 0$ throughout the convection zone and subtracting from the splitting data the contribution from that zone. The latter was computed using a smooth curve drawn through the points in Fig. 3 at radii $> 0.7R$. The optimal averaging procedure was then applied to the truncated kernels

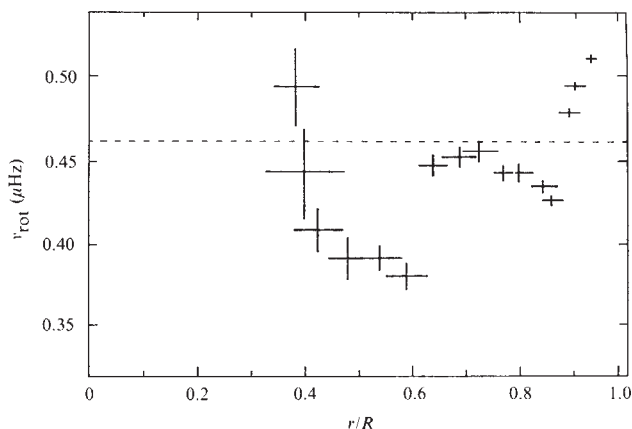


Fig. 3 An optimal-averaging-procedure solution of $\nu_{\text{rot}}(r)$ as a function of fractional radius r/R . The solution below $r = 0.7R$ used a modified procedure discussed in the text. Bar widths indicate the halfwidth of the D kernels and bar heights reflect the standard errors of the observations.

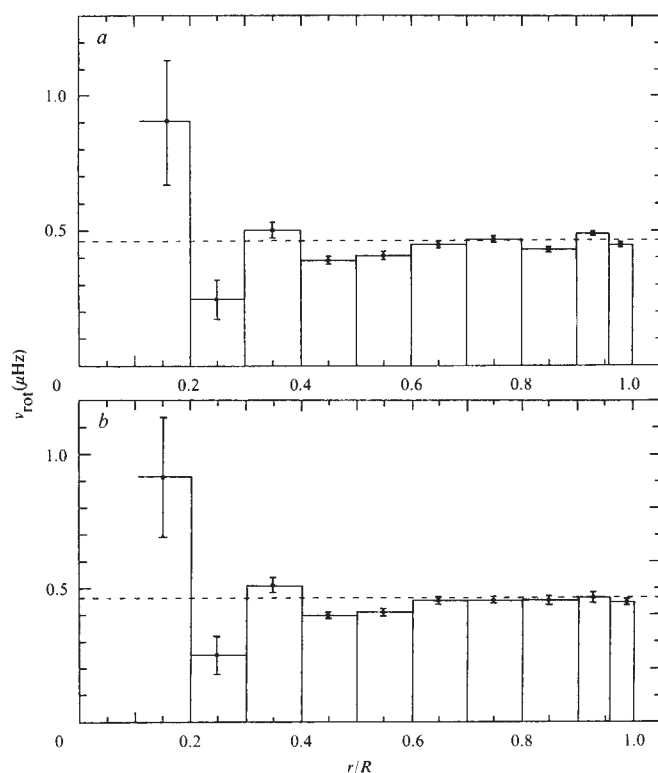


Fig. 4 *a*, A piecewise constant solution of $\nu_{\text{rot}}(r)$ as a function of fractional radius r/R . The fit to the observations was done by minimizing χ^2 . The average error bars indicate the observational uncertainties. *b*, As *a*, except observations of $l = 90$ and 100 were ignored.

and the adjusted data to provide the solution for $r < 0.7R$ shown in Fig. 3. Of course, we could have continued by successively truncating the kernels at smaller and smaller radii, but we did not for fear that the results would be unreliable.

Finally, we represented $\nu_{\text{rot}}(r)$ by a simple, piecewise constant function—a sequence of bins with ν_{rot} constant in each. The inversion of equation (2) then reduces to a set of linear, algebraic equations for the unknown rotation frequencies. We solved the problem by minimizing χ^2 where χ^2 is the sum of the 37 ratios of the square of the difference between each fit and the measured splitting to its variance. Ten bins were used, and the widths of the optimal averaging kernels, D , obtained from the Backus and Gilbert procedure, were used to estimate their relative widths, except for the inner two where the method fails; for these the widths were assigned arbitrarily. Figure 4*a* shows the rotational

frequency by bin and the width of each bin. The bars reflect the uncertainties in the observed splittings, attributing no error to the solar model. Only the outer part of the innermost bin is plotted to emphasize that the rotational splitting kernel is significant only in the outer part of that bin. Figure 4*a* is quite similar to Fig. 3, except at small radii where the optimal averaging procedure fails to give a reliable result. The largest differences between Fig. 4*a* and the polynomial fit of Fig. 2 occur in the deep core where the calculations are most uncertain. All three solutions (Figs 2–4*a*) show much of the interior rotating at or below the equatorial surface rate.

To explore the dependence of the inferred internal rotation shown in Fig. 4*a* on the observed splittings, we minimized χ^2 ignoring the observed $l = 90$ and 100 splittings which differ markedly from the other high-degree data. The only appreciable changes shown in Fig. 4*b* are an increase in the difference between the rotation rates in the two bins nearest the surface and an increase in the uncertainty of those two rates. This emphasizes the important role of the $l = 90$ and 100 splittings in determining the rotation rate near the solar surface. We note that there were no noticeable changes in the deeper rotation rates. Similarly, removal of $l = 1, 2$ and 3 data successively from the solution gave results for $r < 0.4R$ very similar to Fig. 4*a* except with increasing uncertainty. Thus, the largest departures from a rigid rotation rate in Fig. 4*a* arise from the low and high degree splittings; unfortunately, these are the most difficult to measure. Without them, the observations are consistent with rigid rotation, at a statistically significantly slower rate than the equatorial surface value. Thus the three methods yield similar internal rotation. Figure 4*a* is a good representation of the radial variation of rotation that can be inferred from the available data. Measurements of non-sectoral modes would enable us to explore the latitude dependence. Improved measurements of low- and high-degree rotational splitting may significantly change our results for $r < 0.3R$ and $r > 0.9R$.

Discussion

We now consider some of the theoretical implications of our results. Note that some of the structure in our $\nu_{\text{rot}}(r)$ could be due to latitude variations of rotation. However, a latitudinal variation alone cannot explain both the observed surface rotation and low- l splitting measurements that are most sensitive to latitude effects. Many models (reviewed in ref. 16) of the convection zone suggest a relative maximum in $\nu_{\text{rot}}(r)$ near the surface that is consistent with our results. Between 0.9 and $0.6R$ there is little structure in the rotation rate on the resolution scale implied by the data, which implies that there is no significant change in rotation rate at the base of the convection zone. The general flatness of the rotation rate in the convection zone is consistent with the Solberg–Høiland criterion for dynamical stability¹⁷. The nearly constant rotation just beneath the convective envelope is stable to the Goldreich–Schubert–Fricke instabilities^{18,19}. For $r < 0.4R$ our $\nu_{\text{rot}}(r)$ is significantly below the minimum suggested in ref. 20.

The dimensionless measure, J_2 , of the solar gravitational quadrupole moment obtained from the rotation curve in Fig. 4*a* is $(1.7 \pm 0.2) \times 10^{-7}$. The quoted error follows from the observational uncertainties. An additional uncertainty of $\pm 0.3 \times 10^{-7}$ arises from the range of plausible interior models, so the total uncertainty associated with our value is $\pm 0.4 \times 10^{-7}$. The value of J_2 is slightly less than the value for rigid rotation, 1.8×10^{-7} , reflecting that much of the interior is rotating slightly slower than the surface. J_2 was calculated using

$$J_2 = \int_0^R F(r) \nu_{\text{rot}}^2(r) dr \quad (5)$$

where $F(r)$ is the J_2 kernel plotted in ref. 10. Our rotation rate is most uncertain for $r < 0.3R$ and $r > 0.9R$, where $F(r)$ is quite small. By using equation (5), we have assumed that ν_{rot} is independent of latitude throughout the Sun. Thus although our quoted error might be optimistic, we point out, however, that

the dominant contribution to J_2 comes from low latitudes, the region where we have measured ν_{rot} .

According to parametrized, post-newtonian theories of gravitation, the predicted advance of the perihelion of Mercury is

$$\dot{\omega} = 42.95[(2 + 2\gamma - \beta)/3 + 2.9 \times 10^3 J_2] \text{ arc s per } 100 \text{ yr} \quad (6)$$

where γ and β are parameters which are both unity in general relativity. Combining our value of J_2 with the planetary data of Shapiro *et al.*²¹ and that of Anderson *et al.*²², we have

$$(2 + 2\gamma - \beta)/3 = 1.002 \pm 0.005 \text{ and } 1.006 \pm 0.005 \quad (7)$$

Our value of J_2 makes an essentially negligible contribution to equation (7). In particular, the contribution of the J_2 term from equation (6) to equation (7) is a factor of 10 less than the errors quoted in equation (7), which are due to the planetary data. Therefore, at the present level of accuracy of the planetary data our value of J_2 is not in conflict with general relativity.

As noted above, there is an apparent discrepancy between observational estimates of the internal rotation. We calculate a markedly slower internal rotation rate than Hill *et al.*⁹. The discrepancies between the two results are much larger than the measurement uncertainties appear to allow. Unfortunately, there are no modes common to both data sets, so a direct comparison of splittings is not possible. The theory by which the interior

rotation rate is calculated from a set of rotational splittings is sufficiently well understood that the two different sets of splittings should yield comparable rotation rates, at least if ν_{rot} is a slowly varying function of r .

It is mathematically possible (see ref. 23) to find a function ν_{rot} that is consistent with all of the data, but we find it implausible that the details of the rapid rotation variation that would then be required would be such as to impart high rotational splitting just to the modes that happen to have been identified in one set of observations. This suggests that there are systematic errors in at least one set of results. The most likely source of systematic errors is mode misidentification, that is, either a feature in a spectrum that is not a mode is wrongly identified as a mode or a feature is assigned incorrect values of n, l, m . The relative simplicity and high resolution in l in the spectra of Duvall and Harvey¹ compared with Bos and Hill⁸, plus the unexpected appearance (Fig. 1 of ref. 9) of zonal modes in the latter analysis, lends confidence to the present work.

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- Duvall, T. L. Jr & Harvey, J. W. *Nature* **310**, 19–22 (1984).
- Cowling, T. G. & Newing, R. A. *Astrophys. J.* **109**, 149–158 (1949).
- Ledoux, P. *Astrophys. J.* **144**, 373–384 (1951).
- Claverie, A., Isaak, G. R., McLeod, C. P., van der Raay, H. B. & Roca Cortes, T. *Nature* **293**, 443–445 (1981).
- Isaak, G. R. *Nature* **296**, 130–131 (1982).
- Dicke, R. H. *Astrophys. J.* **228**, 898–902 (1979).
- Woodard, M. & Hudson, H. *Bull. Am. astr. Soc.* **15**, 951–952 (1983).
- Bos, R. J. & Hill, H. A. *Sol. Phys.* **82**, 89–102 (1983).
- Hill, H. A., Bos, R. J. & Goode, P. R. *Phys. Rev. Lett.* **49**, 1794–1797 (1982).
- Gough, D. O. *Nature* **298**, 334–339 (1982).
- Delache, P. & Scherrer, P. *Nature* **306**, 651–653 (1983).
- Hansen, C. J., Cox, J. P. & Van Horn, J. M. *Astrophys. J.* **217**, 151–159 (1977).
- Gough, D. O. *Mon. Not. R. astr. Soc.* **196**, 731–745 (1981).
- Christensen-Dalsgaard, J., Gough, D. O. & Morgan, J. G. *Astr. Astrophys.* **73**, 121–128 (erratum **79**, 260) (1979).
- Backus, G. & Gilbert, F. *Phil. Trans. R. Soc. A266*, 123–192 (1970).
- Gilman, P. A. in *Physics of the Sun* (Reidel, Dordrecht, in the press).
- Zahn, J.-P. *IAU Symp.* **59**, 187–194 (1974).
- Goldreich, P. & Schubert, G. *Astrophys. J.* **150**, 571–587 (1967).
- Fricke, K. Z. *Astrophys. J.* **68**, 317–344 (1968).
- Spruit, H. C., Knobloch, E. & Roxburgh, I. W. *Nature* **304**, 520–522 (1983).
- Shapiro, I. I., Counselman, C. C. & King, R. W. *Phys. Rev. Lett.* **36**, 555–558 (1976).
- Anderson, J. D., Keeseey, M. S. W., Lau, E. L., Standish, E. M. & Newhall, X. X. *Acta astronaut.* **5**, 43–61 (1978).
- Gough, D. O. *Mem. Soc. astr. Ital.* (in the press).

Sequence of a *Drosophila* segmentation gene: protein structure homology with DNA-binding proteins

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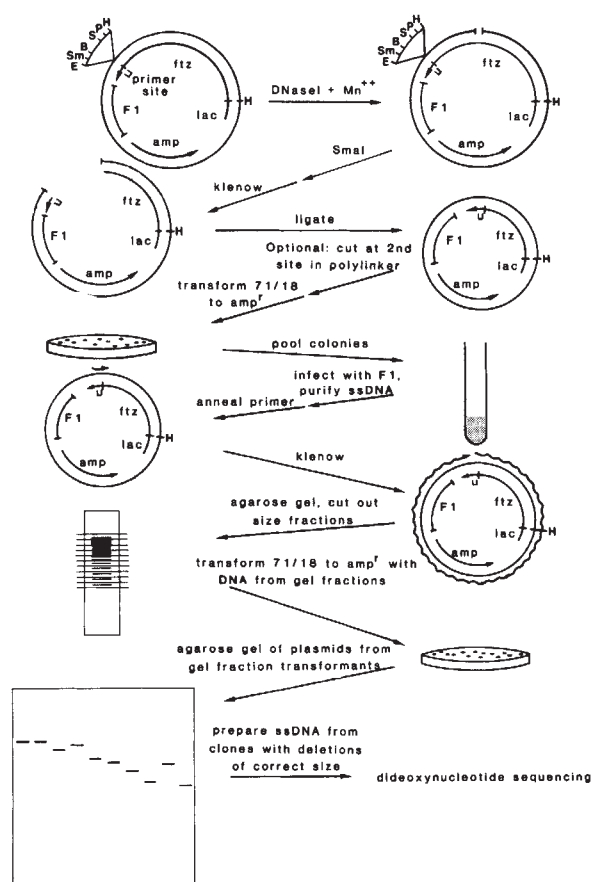
Mutations in the fushi tarazu (ftz) locus of Drosophila result in embryos with half the usual number of body segments. The sequences of the wild-type gene, a temperature-sensitive allele and a dominant mutant allele are presented. A portion of the conserved protein domain present in ftz and several homoeotic genes resembles the DNA-binding region of prokaryotic DNA-binding proteins, and is also similar to products of the yeast mating-type locus.

Two well-defined processes in the development of *Drosophila melanogaster* are the establishment of a segmented body pattern and the specification of segmental identity (see ref. 1 for discussion). A large number of genes involved in establishing proper segmentation have been identified^{2–7}. The homoeotic genes of the bithorax complex (BX-C) are involved in determining the identity of thoracic and abdominal segments^{8–10}. A second cluster of genes, the antennapedia complex (ANT-C)¹¹, functions primarily in head and thoracic development and contains homoeotic loci, for example *Antennapedia* (*Antp*), *Sex combs reduced* (*Scr*) and *proboscipedia* (*pb*), and a segmentation gene *fushi tarazu* (*ftz*)³. Recessive lethal *ftz* mutant alleles result in embryos having half the normal number of segments. This phenotype has also been interpreted⁴ as having homoeotic character as the pairwise fused segments appear to have the

identity of the more anterior segment, that is, (T1 + T2) → T1, (T3 + A1) → T3, etc. Temperature shift experiments with a temperature-sensitive allele, *ftz*^{f4715}, indicate that *ftz* expression is required only during the cellular blastoderm stage between 2 and 4 h after fertilization⁴; this is the time at which *ftz* transcripts are most abundant¹², long before segments become visible, suggesting that *ftz* functions in establishment but not maintenance of the pattern.

Molecular characterization of the ANT-C has included identification of the *Antp* and *ftz* transcript units^{12–14}. One important finding is that the coding regions of the 3' exons of *Antp* and *ftz* and of the *Ultrabithorax* (*Ubx*) gene of the BX-C share an ~180-base pair (bp) (60-codon) region of homology^{13–16}. Over 90% of the amino acid sequences in this region are conserved¹⁶. The amino acid composition of the region is 28–30% lysine and

Fig. 1 Outline of a method for generating staggered DNA subclones for dideoxynucleotide sequencing. The key to this procedure is that relatively large DNA inserts (up to 6 kb in our experience) are stable in pEMBL single-stranded plasmids³⁸, which has allowed us to adopt the sequencing strategy of Frischauf *et al.*³⁹ for use with dideoxynucleotide sequencing. pEMBL plasmids contain the F1 replication origin and are replicated as single strands and packaged as phage when F1 infection provides replication functions in *trans*³⁸. DNA purification and subsequent enzymatic steps were performed according to standard protocols⁴⁰. Preparation of single-stranded DNA was as described elsewhere³⁸. DNA to be sequenced (in this case a 3.5-kb *Hind*III fragment containing *ftz*) is cloned into a site in the pEMBL polylinker as far as possible from the primer site (in this case, the *Hind*III site of pEMBL9⁺). An average of one random or quasi-random cut per molecule is made with DNase I in the presence of Mn^{2+} (ref. 41) or with restriction enzymes. A second cut is introduced with a restriction enzyme that cleaves as close to the primer site as possible, but which fails to cleave the insert DNA (*Sma*I in this case). The ends of the DNA are repaired with the Klenow fragment of DNA polymerase I followed by ligation of the DNA at concentrations which favour recircularization ($10 \mu\text{g ml}^{-1}$). Thus a series of deletions is generated, each having one end at the polylinker site. To eliminate clones deleted in the direction opposite to the insert DNA, the ligated DNA is cleaved with a second enzyme which cuts only at a site in the polylinker between the first restriction site and the insert DNA. DNAs with deletions extending in the wrong direction are cleaved while those with deletions into the insert (thus lacking this second restriction site) remain uncut. The DNA is then used to transform *E. coli* strain 71-18 (ref. 38), selecting for ampicillin resistance (Amp^r)²³. Circular molecules transform at a much higher frequency than linear ones, eliminating most of the clones which have deletions extending in the wrong direction. About 500 Amp^r colonies are pooled in liquid culture and infected with F1 strain IRI. Single-stranded F1 and pEMBL-derived DNA is purified from the resulting phage mixture. This step selects against deletions which destroy the F1 replication origin of the pEMBL plasmids. The mixture of single-stranded DNAs are then converted to double strands by annealing of the pentadecamer sequencing primer (New England Biolabs) and synthesis of the second strand with Klenow fragment. 2 ng of primer are annealed to 0.1 μg single-stranded DNA in 15 μl of 20 mM Tris-HCl pH 8.5, 10 mM $MgCl_2$ at 42 °C for 1 h; to this mixture is then added 2 μl of each 2 mM dXTP, 1 μl of 0.1 M dithiothreitol, 4 μl H_2O , 0.5 μl Klenow (5 units μl^{-1}). The reaction is carried out at 37 °C for 30 min, after which an additional 0.5 μl of Klenow is added, followed by 30 min more at 37 °C. The DNA is then fractionated in a 0.7% low-melting temperature agarose gel and slices between the sizes of pEMBL (deletion of entire insert) and pEMBL+insert (not deleted) cut out. Only DNAs which contain the primer site will be converted to double-stranded molecules, again selecting against deletions extending in the wrong direction. DNA is extracted from the gel slices and used to transform strain 71-18, selecting for ampicillin resistance. The sizes of deletions in individual Amp^r clones are determined by agarose gel electrophoresis of double-stranded plasmid DNAs. Dideoxynucleotide sequencing is done using single-stranded DNA from clones with deletion end points 200–300 bp apart (the length of sequence determined per end being 250–350 bp).



arginine; the region may be involved in DNA or RNA binding. This region of homology also hybridizes with several other positions within the ANT-C and BX-C, suggesting that the genes of the two complexes are functionally and evolutionarily related and that the region of homology defines a common function or property of the products encoded by these complexes. In particular, it suggests a relationship between at least some homoeotic (*Antp* and *Ubx*) and segmentation (*ftz*) loci.

The *ftz* gene has been delimited to a 3.2-kilobase (kb) DNA fragment (3.5 kb in some *ftz*⁺ chromosomes)¹²; this fragment contains both a 1.8-kb transcript and the two molecular lesions thus far associated with *ftz* mutations (a 4.9-kb insertion and a translocation breakpoint). Here we report the DNA sequences of three *ftz* alleles (the wild type and two mutations) and discuss the implications of this sequence information for the function of *ftz* and related genes.

Structure of the *ftz* gene

Clones for dideoxynucleotide sequencing of *ftz* were constructed as described in Fig. 1 legend. The regions sequenced are summarized in Fig. 2 and the nucleotide sequences of *ftz* genomic and cDNA clones and of *ftz*^{f47ts} and *ftz*^{Rp1} mutations are given in Fig. 3. The *ftz* genomic clone contains a 1,239-bp open reading frame in the direction of transcription which is split by a 150-bp intron containing stop codons in all three frames. The cDNA clone does not contain this intron. Transcription starts at position -191 as determined from primer extension experiments (data not shown). The -191 start point is also

consistent with the structure of the cDNA (see below). Two 'TATA' boxes occur 21 and 31 bp upstream from the transcriptional start; the latter is the more conventional spacing. A series of 'AATAAA' poly(A) addition site sequences occur immediately downstream from the 3' end of the cDNA, but no data are available to determine which of these is used. A transcript ending within the series of polyadenylation sites, with the intron spliced out, would be approximately 1.8 kb in size, in agreement with the size of the previously reported *ftz* RNA¹².

Structure of the *ftz* cDNA clone

The first 91 bp at the 5' end of the cDNA sequence do not correspond to the genomic sequence (Fig. 3). The structure of the 5' end of the cDNA clone could have arisen if, during cDNA synthesis, an inverted repeat complementary to the 5' end of the transcript (Fig. 3) formed a hairpin structure in the first cDNA strand (Fig. 4). The hairpin could then serve to prime second strand synthesis. *S*₁ nuclease was used to remove the foldback loop during preparation of the library and the observed cDNA 5' end would have been created by *S*₁ nuclease cleavage at the site indicated in Fig. 4. The cloning of the cDNA was completed by ligation of *Eco*RI linkers, transformation into *Escherichia coli*, and apparent subsequent resolution of the heteroduplex in favour of the bold-lettered strand in Fig. 4. As synthesis of cDNA libraries commonly relies on self-primed synthesis of the second cDNA strand, it is likely that many cDNAs will contain such anomalous sequences at their 5' ends. Another example has been observed in a *Ubx* cDNA clone from

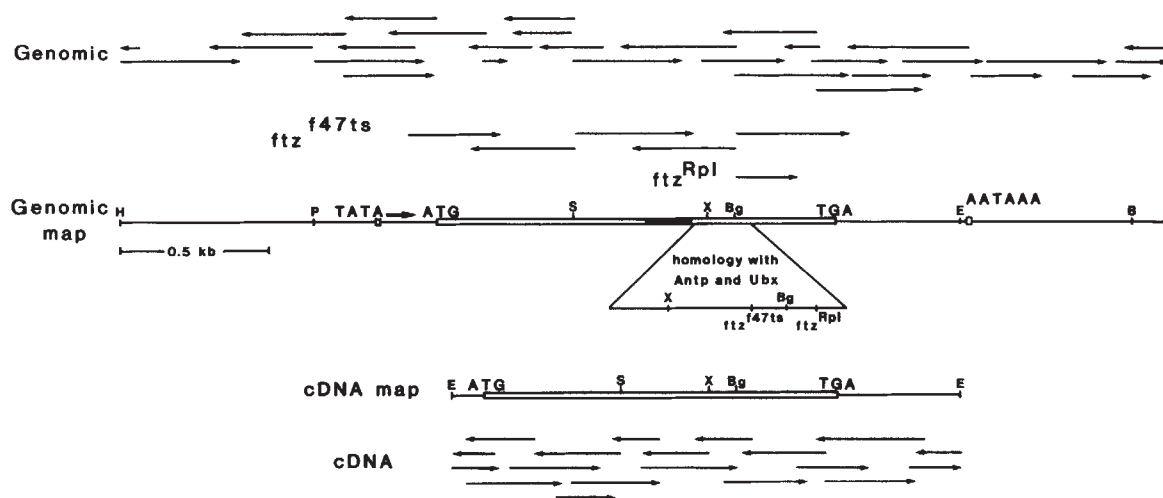


Fig. 2 Summary of dideoxynucleotide sequencing of wild-type and mutant *ftz* genomic clones and a *ftz* cDNA clone. Deleted clones for sequencing were derived as described in Fig. 1 legend, except for the sequences of *ftz*^{f47ts} and *ftz*^{Rp1} in which deletions were made from specific restriction sites. The genomic clone is a 3.5-kb *Hind*III fragment from a *ftz*⁺ P^P chromosome¹². The *ftz*^{f47ts} mutation was induced on this same chromosome. The *ftz* cDNA clone was isolated as a 1.7-kb *Eco*RI fragment from the Oregon R, 0–5-h embryonic cDNA library of Goldschmidt-Clermont, Saint and Hogness (personal communication). Genomic and cDNA sequencing runs are shown above and below the maps of these clones respectively. Open bar, coding region; solid bar, intron. The positions of TATA and AATAAA boxes are shown; arrow, start of transcription. The region of homology with *Antp* and *Ubx*¹⁶ is shown expanded, as are the positions of the *ftz*^{f47ts} point mutation and the *ftz*^{Rp1} (refs 2,3) reciprocal translocation breakpoint: material to the right (distal) of the *ftz*^{Rp1} breakpoint is from chromosome 2, or may be a small insertion of unknown origin¹². B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; P, *Pst*I; S, *Sal*I; X, *Xho*I. Single-stranded DNA was prepared for sequencing as described by Biggin *et al.*⁴² except that reactions were done in the wells of microtitre plates (D. Peattie, personal communication).

the same library (M. Goldschmidt-Clermont, R. Saint and D. S. Hogness, personal communication). The secondary structure at the 5' end of the *ftz* mRNA could have a function relating to the translation, processing, localization or stability of the RNA.

Encoded proteins

The first AUG in the *ftz* transcript is 24 bp in from the 5' end at position -97 in Fig. 3; translation starting at this AUG would result in a 22-amino acid peptide ending at a UAG triplet. This reading frame is out of register with the main 1,239-bp *ftz* open frame. The context of adjacent nucleotides at the -97 AUG has been observed only once in initiator AUGs but is found 14 times in 'nonfunctional' upstream AUGs¹⁷. However, the codon usage of this short open reading frame is biased correctly for *D. melanogaster* coding regions (P. O'Connell and M. Rosbash, personal communication), suggesting that it may be functional. As the 22-codon open reading frame is within a hairpin loop of the RNA (Fig. 4), its translation might influence RNA secondary structure. It will be necessary to generate mutations within the 22-codon region to determine whether it is functional. The second AUG in the *ftz* transcript is the beginning of the 1,239-bp open reading frame (position 1). The second AUG may also be involved in base pairing if the secondary structure shown in Fig. 4 exists in the mRNA. The third AUG at position 46 is also in the correct frame and is not included in the inverted repeat at the 5' end of the *ftz* transcript. Translation starting at position 1 would yield a 413-amino acid protein of molecular weight (MW) 45,000 while initiation at position 46 would yield a 398-amino acid protein of MW 43,500.

By several criteria the *ftz* protein seems to be divided into three domains. The 189-bp *Antp*, *ftz* and *Ubx* homology region begins 8 bp downstream from the 150-bp intron in *ftz* and the break in homology at the 3' end is abrupt. Of the residues encoded by the homology region, 30.6% are basic while the two remaining portions of the protein at the amino and carboxyl ends are 9.9% and 10.4% basic, respectively. Thus, the protein seems to be divided into relatively neutral amino-terminal and carboxy-terminal domains with a very basic homology region in between. In addition, the amino and carboxyl domains each comprise more than 10% proline and 10%

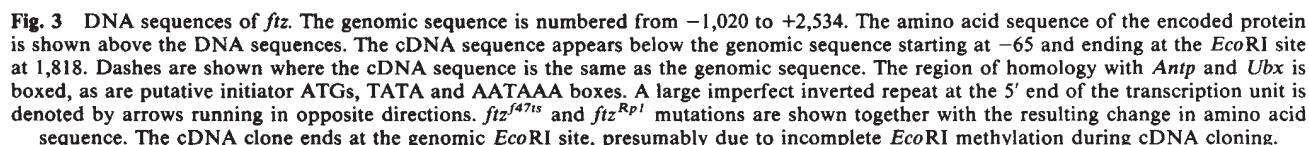
tyrosine and the carboxyl domain comprises almost 15% glutamine. The homology domain is not rich in any of these amino acids.

Comparison of the *ftz* genomic DNA sequence with that of the cDNA clone (isolated from the Oregon R cDNA library of Goldschmidt-Clermont, Saint and Hogness) reveals six sequence polymorphisms (Fig. 3): four of these are in the third base of a codon and do not change the amino acid sequence. Another third-position difference at nucleotide 1,344 places a histidine, rather than a glutamine, in the cDNA. The sixth polymorphism is the deletion of 9 bp in the cDNA clone encoding Tyr-His-Ser at nucleotides 139–147 in the genomic clone.

Structure of mutant *ftz* alleles

The *ftz* locus was first mapped by using chromosome rearrangements associated with *ftz* mutations¹². One of the mutations, *ftz*^{Rp1}, originally found by I. Duncan, is associated with a translocation of parts of chromosomes 2 and 3. The *ftz*^{Rp1} allele is of particular interest because it has both a recessive lethal *ftz* phenotype and a dominant *postbithorax*-like phenotype. The *postbithorax* (*pbx*) phenotype, in which posterior third thoracic structures are transformed into posterior second thoracic structures, is normally a recessive phenotype resulting from mutations in the *pbx* locus of the bithorax complex. Flies that are *pbx*/*pbx* and flies that are *ftz*^{Rp1}/+ both have posterior halteres transformed into posterior wings (that is, posterior T3 → posterior T2). The dominant effect of *ftz*^{Rp1} cannot be due to loss of *ftz* function, as flies with a deletion of *ftz* heterozygous with a wild-type *ftz* allele develop normally. Therefore, the dominance of *ftz*^{Rp1} is due to abnormal activity of *ftz*—this is consistent with the partial loss of function recessive lethal phenotype of *ftz*^{Rp1}.

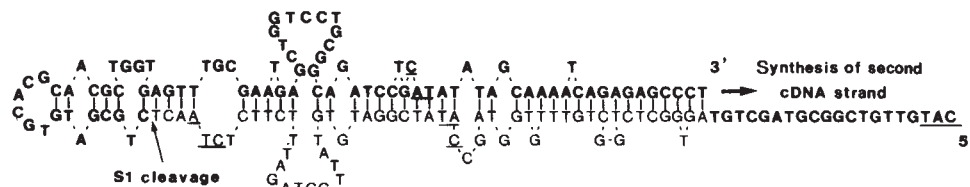
A restriction map of the *ftz*^{Rp1} allele showed the breakpoint within the *ftz* gene to be near the 3' end¹². The relevant part of the mutant gene was sequenced (Fig. 3) and reveals that *ftz*^{Rp1} encodes a truncated protein in which the C-terminal 100 amino acids of the normal protein have been replaced by 10 novel amino acids. The breakpoint of the chromosome rearrangement is within the sequence that is similar to the other homoeotic loci. The homology region is largely but not completely intact in the *ftz*^{Rp1} protein product. It seems likely that the shortened



Crystallographic structures determined for the cro and repressor proteins of bacteriophage λ and the CAP protein of *E. coli* have revealed the existence of conserved α -helical structures which computer-modelling studies suggest are the DNA-binding sites for these proteins¹⁸⁻²⁴. The model is supported by studies of protection from chemical modification by binding of these pro-

Fig. 4 Formation of the *ftz* cDNA. The figure shows a hypothetical secondary structure of a cDNA strand complementary to the 5' end of the *ftz* transcript. Nucleotides shown in bold letters correspond to the observed sequence of the cDNA clone. The 3' end of the hairpin structure presumably serves as primer for the synthesis of the second cDNA strand.

S_1 nuclease cleavage at the indicated site followed by ligation of *EcoRI* linkers would give rise to the observed cDNA sequence if the mismatched sequences were resolved by copying the bold-lettered strand. The underlined codon triplets are complementary to the 'upstream' AUG which would begin a 22-amino acid leader peptide, the UAG ending this open reading frame, and the two possible initiator AUGs for the major *ftz* open reading frame.



teins to their operator DNAs²⁴ and by extensive genetic analysis^{25,26}. The DNA-binding structure (reviewed in ref. 24) comprises two α -helices: 'helix 3' fits into the major groove of DNA and 'helix 2' lies across helix 3, holding it in position. The two helices are connected by a β -turn. The amino acids which are important for the conformation of this structure are conserved in these proteins and in homologous proteins from other lambdoid phages as summarized in Fig. 5. Alanine is usually found in position 7, isoleucine or valine in position 17, and hydrophobic residues are found in positions 6, 10, 12 and 20. The side chains at positions 10–12 are small to permit a tight turn between the helices. Amino acids in positions 13–16, 18, 19, 21 and 22 are involved in determining DNA sequence specificity (other parts of the protein can have some influence also). Similar patterns of conserved amino acids have been found in several bacterial regulatory proteins and in the *MATa1* gene of yeast (Fig. 5)^{21–24,27}. The position of the DNA-binding domain within the bacterial proteins varies (Fig. 5).

The conserved pattern of amino acids found in bacterial DNA-binding proteins is also present near one end of the conserved amino acid sequence common to *ftz*, *Antp* and *Ubx* (Fig. 5). The *ftz*, *Antp* and *Ubx* sequences are also similar to the putative DNA-binding regions encoded by the yeast *MATa1* and *MATa2* genes (19/29 amino acids are homologous to *MATa1*, 13/29 amino acids are homologous to *MATa2*). Similarity between *MATa2* and the *ftz*, *Antp* and *Ubx* homology regions was originally noted by Shepard *et al.*²⁸. The homologous *Drosophila* and yeast sequences shown in Fig. 5 are flanked by very basic regions that are unlikely to form α -helices. The conserved glycine residue following helix 2 is replaced by serine in *ftz* and by cysteine in *Antp* and *Ubx*; this glycine is thought to be important for formation of the tight turn necessary for the two helices to come into close contact. However, a glutamic acid residue can be accommodated at this position in a functional λ repressor²⁹. In addition, serine and cysteine are the only substitutions for glycine at this position in putative DNA-binding structures from other bacterial proteins (Fig. 5).

Further evidence that the possible DNA-binding region of *ftz* is in fact important for the function of the protein comes from the temperature-sensitive mutation described above. The *ftz*^{f47is} mutation changes the highly conserved alanine in helix 2 to a valine. The alanine normally comes in close contact with the conserved isoleucine/valine in helix 3 (ref. 24). Although the Ala $\rightarrow$ Val change is fairly conservative, the added bulk of the valine side chain would be predicted to increase the distance between the two helices, perhaps destabilizing the protein in this region. It would be instructive to know the phenotype of a mutation affecting this alanine in λ repressor, *cro* or *lac* repressor (in which many mutations have been sequenced^{25,26,30}), but unfortunately no mutations at this position have been reported. The *ftz*^{Rp1} translocation breakpoint occurs exactly at one end of the homology with *MATa1* and *MATa2* and probably results in an abnormally functioning protein.

According to the model outlined above, the specificity of DNA binding would be determined by the side groups protruding from the α -helix which lies in the DNA major groove (helix 3 in Fig. 5). The sequences of *ftz*, *Antp* and *Ubx* are identical in this region, suggesting that they might recognize and bind to the same DNA sequence.

Conclusions

The extraordinary size and complexity of some of the *Drosophila* homoeotic genes, for example the 73-kb *Ubx* unit³¹ and the 103-kb *Antp* unit^{13,14}, contrasts with the relative simplicity of the *ftz* gene¹². Yet *ftz* shares a sequence encoding a protein domain of ~6,500 MW with *Antp* and *Ubx*¹⁶. The *ftz*^{Rp1} allele provides another indication of related function. This *ftz* allele results in a phenotype characteristic of a class of mutations in the bithorax complex. The recessive *ftz* phenotype (pairwise segmental fusions) sets *ftz* apart from the classical homoeotic genes, in which mutations generally cause dramatic transformations of only one to a few body segments. The function of *ftz*⁺ seems to be to determine the formation of alternate segments; presumably other homoeotic genes further specify the identities of the segments. The location of *ftz* only 30 kb from the homology domain of *Antp* suggests an evolutionary relationship between the *Antp* 3' exon and the *ftz* 3' exon¹⁶.

It has been clear from genetic experiments, and has been confirmed by *in situ* hybridization to sectioned tissues^{32–35}, that there is specific expression of *Drosophila* regulatory genes in particular regions of the embryo. The *in situ* hybridization experiments demonstrate that expression is controlled at the level of RNA synthesis or stability, which leads to the question: how is spatial regulation of RNA accumulation attained? A second question is how the regulatory genes direct the development of particular structures in lieu of the alternative structures that develop when the genes are mutated. The structure of the protein encoded by *ftz*, and of structurally related proteins including products of *Antp* and *Ubx*, indicate that DNA binding may be an important aspect of the function of genes that control segmentation.

The data presented above suggest that the protein structure common to *ftz*, *Antp* and *Ubx* products includes a DNA-binding region of the type observed in some bacterial proteins. The primary (but not the only) part of the bacterial proteins involved in DNA sequence recognition is the helix 3 domain. Note that the amino acid sequences of *Antp*, *Ubx* and *ftz* products are identical in the region of the homoeotic homologue of helix 3, and in flanking regions. The one exception is a serine residue in *ftz*, just N-terminal to helix 3, in the position occupied by threonine in *Antp*, *Ubx*, *MATa1* and *Fnr* (Fig. 5). The near identity of the homoeotic gene products in this critical region suggests that they may bind to similar or identical DNA sequences, and may in fact compete with each other during early embryogenesis. Such competition suggests a mechanism behind mutations such as *ftz*^{Rp1}, in which a BX-C recessive loss of function phenotype (*pbx*) results from a dominant *ftz* mutation. The truncated *ftz*^{Rp1} protein product may compete with or otherwise influence the DNA binding of BX-C products. The binding affinities could also be dependent on cooperative or competitive interactions with other proteins. It is also possible that *ftz* regulates expression of ANT-C or BX-C genes.

There are many other genes that are not obviously homoeotic in which mutations alter the number or pattern of segments that develop^{2,5–7}. DNA hybridization experiments suggest that the region of homology common to *ftz*, *Antp* and *Ubx* cannot be present at most of the other segmentation loci (ref. 15 and unpublished results). However, it is possible that a similar

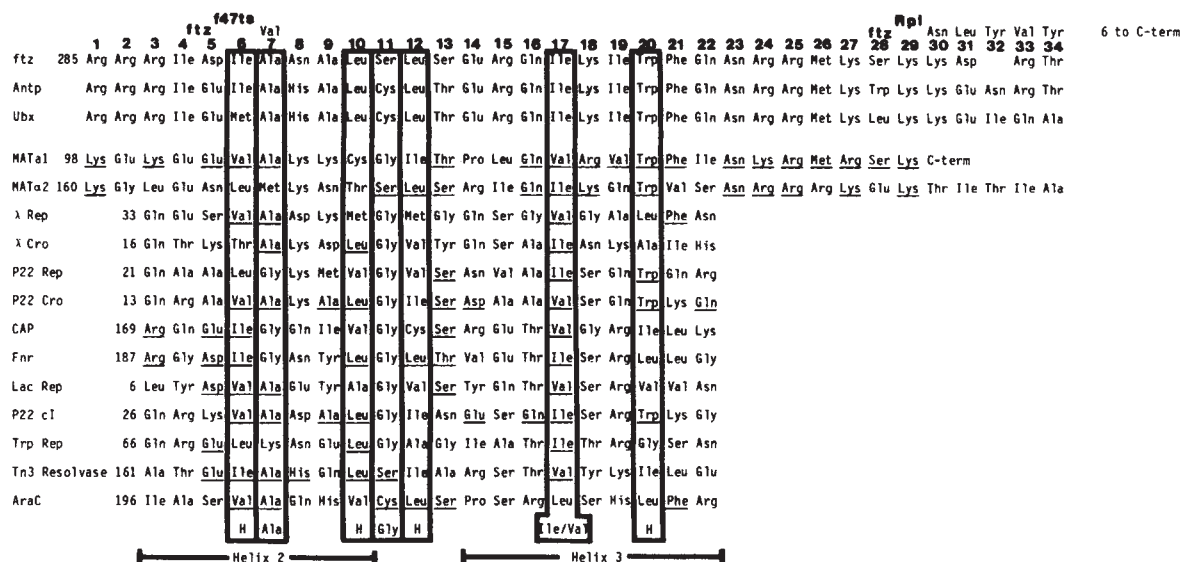


Fig. 5 Putative DNA-binding domain within the homoeotic homology region. *ftz*, *Antp* and *Ubx* sequences at the 3' end of their shared homology are aligned with prokaryotic and potential yeast DNA-binding domains²⁴. The sequence of the yeast *MATA1* protein past the tryptophan at position 118 is different from that reported previously due to previously undiscovered *MATA1* introns (K. Nasmyth, personal communication). Sequence changes due to *ftz* mutations are shown at the top. The positions of the two α -helices of the DNA-binding domain (numbered 2 and 3 according to convention for the λ *cro* protein) are shown at the bottom. Conserved residues within the domain, important for close alignment of the two helices, are boxed. Amino acids that are structurally homologous to *ftz*, *Antp* or *Ubx* residues are underlined. The number to the left of each sequence is the position of the first amino acid shown within the complete protein. The positions of the amino acids are arbitrarily numbered at the top (1–34). The collection of bacterial sequences shown is from a review by Pabo and Sauer (ref. 24 and references therein). Yeast sequences are from ref. 43 and K. Nasmyth (personal communication); *Drosophila* sequences are from ref. 16.

domain is encoded by some of the other loci but is sufficiently divergent in sequence to be recognizable only when the gene, or its protein, has been sequenced.

If part of the homology region of the homoeotic genes encodes a DNA-binding domain, what could be the function of the rest of the homology region? The most striking aspect of the remaining sequence is its basicity: arginine and lysine comprise about 30% of the amino acids in the homology domain¹⁶. Very basic sequences are also present in the C-terminal parts of *Antp* and *Ubx*, just downstream from the homology region (M.P.S., unpublished data). Such a basic protein sequence is suggestive of nonspecific DNA or RNA binding; domains known to be involved in specific DNA binding are not especially basic.

There are important limitations to the correlations we have made between the structure of the homoeotic gene products and the bacterial DNA-binding proteins: (1) Only three of the bacterial proteins have been crystallized^{18–20}, so the full range of possible variations in the structure of the DNA-binding helix-turn-helix structure is unknown. (2) It is unknown whether the parts of the two yeast mating-type protein products that are homologous with the bacterial proteins are in fact used for DNA binding. (3) Some known DNA-binding proteins apparently lack this type of binding sequence^{36,37}. However, the similarity of the *Drosophila* sequences to the bacterial sequences is striking, and the type of helix-turn-helix structure found in these DNA-binding proteins has not been observed in proteins which are not DNA-binding proteins. It has also been shown that the *MATA2* product is a sequence-specific DNA-binding protein (A. Johnson and I. Herskowitz, personal communication). Thus, we believe that the structural homology between the homoeotic gene products and known DNA-binding proteins is strong enough to suggest that the homoeotic genes may, at least in part, function through DNA-binding mechanisms.

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- Raff, R. A. & Kaufman, T. C. *Embryos, Genes and Evolution* (Macmillan, London, 1983).
- Nusslein-Volhard, L. & Wieschaus, E. *Nature* **287**, 795–801 (1979).
- Wakimoto, B. T. & Kaufman, T. C. *Dev. Biol.* **81**, 51–64 (1981).
- Wakimoto, B. T., Turner, F. R. & Kaufman, T. C. *Dev. Biol.* **102**, 147–172 (1984).
- Nusslein-Volhard, C., Wieschaus, E. & Kluding, H. *Wilhelm Roux Arch. dev. Biol.* (in the press).
- Jurgens, G., Wieschaus, E., Nusslein-Volhard, C. & Kluding, H. *Wilhelm Roux Arch. dev. Biol.* (in the press).
- Nusslein-Volhard, C. & Jurgens, G. *Wilhelm Roux Arch. dev. Biol.* (in the press).
- Lewis, E. B. *Cold Spring Harb. Symp. quant. Biol.* **16**, 159–174 (1951).
- Lewis, E. B. *Nature* **276**, 565–570 (1978).
- Bender, W. et al. *Science* **221**, 23–29 (1983).
- Kaufman, T. C., Lewis, R. & Wakimoto, B. *Genetics* **94**, 115–133 (1980).
- Wiener, A. J., Scott, M. P. & Kaufman, T. C. *Cell* (in the press).
- Scott, M. P. et al. *Cell* **35**, 736–776 (1983).
- Garber, R. L., Kuroiwa, A. & Gehring, W. J. *EMBO J.* **2**, 2027–2036 (1983).
- McGinnis, W., Levine, M. S., Hafen, E., Kuroiwa, A. & Gehring, W. J. *Nature* **308**, 428–433 (1984).
- Scott, M. P. & Weiner, A. J. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Kozak, M. *Microbiol. Rev.* **47**, 1–45 (1983).
- Anderson, W. F., Ohlendorf, D. H., Takeda, Y. & Matthews, B. W. *Nature* **290**, 754–758 (1981).
- McKay, D. B. & Steitz, T. A. *Nature* **290**, 744–749 (1981).
- Pabo, C. O. & Lewis, M. *Nature* **298**, 443–447 (1982).
- Matthews, B. W., Ohlendorf, D. H., Anderson, W. F. & Takeda, Y. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1428–1432 (1982).
- Sauer, R. T., Yocum, R. R., Doolittle, R. F., Lewis, M. & Pabo, C. O. *Nature* **298**, 447–451 (1982).
- Weber, I. T., McKay, D. B. & Steitz, T. A. *Nucleic Acids Res.* **10**, 5086–5102 (1982).
- Pabo, C. O. & Sauer, R. T. *A. Rev. Biochem.* (in the press).
- Nelson, H. C. M., Hecht, M. H. & Sauer, R. T. *Cold Spring Harb. Symp. quant. Biol.* **47**, 441–449 (1983).
- Hecht, M. H., Nelson, H. C. M. & Sauer, R. T. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2676–2680 (1983).
- Matthews, B. W., Ohlendorf, D. H., Anderson, W. F., Fisher, R. G. & Takeda, Y. *Cold Spring Harb. Symp. quant. Biol.* **47**, 427–433 (1983).
- Shepherd, J. C. W., McGinnis, W., Carrasco, A. E., De Robertis, E. M. & Gehring, W. J. *Nature* **310**, 70–71 (1984).

29. Hochschild, A., Irwin, N. & Ptashne, M. *Cell* **32**, 319–325 (1983).
30. Miller, J. H. in *The Operon* (eds Miller, J. H. & Reznikoff, W.) 31–88 (Cold Spring Harbor Laboratory, New York, 1978).
31. Akam, M. *Trends biochem. Sci.* **8**, 173–177 (1983).
32. Akam, M. *EMBO J.* **2**, 2075–2084 (1983).
33. Levine, M., Hafen, E., Garber, R. L. & Gehring, W. J. *EMBO J.* **2**, 2037–2046 (1983).
34. Hafen, E., Levine, M. & Gehring, W. J. *Nature* **307**, 287–289 (1984).
35. Hafen, E., Kuroiwa, A. & Gehring, W. J. *Cell* (in the press).
36. Battey, J. et al. *Cell* **34**, 779–787 (1983).

37. Fiers, W. et al. *Nature* **273**, 113–120 (1978).
38. Dente, L., Cesareni, G. & Cortese, R. *Nucleic Acids Res.* **11**, 1645–1655 (1983).
39. Frischauf, A. M., Garoff, H. & Lehrach, H. *Nucleic Acids Res.* **8**, 5541–5549 (1980).
40. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
41. Heffron, F., So, M. & McCarthy, B. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6012–6016 (1978).
42. Biggin, M. D., Gibson, T. J. & Hong, G. F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3963–3965 (1983).
43. Nasmyth, K. A., Tatchell, K., Hall, B. D., Astell, C. & Smith, M. *Cold Spring Harb. Symp. quant. Biol.* **45**, 961–981 (1980).

LETTERS TO NATURE

Nature of blue galaxies in the cluster C11447+2619

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Photometric studies of distant clusters of galaxies provide evidence for significant evolution of the member galaxies during the relatively recent cosmological past^{1–5}. Here, spectroscopy is presented that confirms a previous photometric result that the distant ($z = 0.38$) cluster C11447+2619 contains the largest excess of blue galaxies yet encountered. The galaxy surface density profile of the cluster shows it to be open and irregular, making it the first such cluster to be studied at high redshift. The spectra indicate that the blue colours are predominantly the result of recent star formation. We speculate that vigorous star formation in galaxies, independently of their environments, was much more common several Gyr ago than it is in the Universe today.

We have discussed elsewhere³ broad-band photometric data on 33 nearby and distant (to $z = 0.54$) clusters of galaxies and the nearby field. That sample was chosen from the literature to include as many large redshift clusters as were known to be very rich and to have at least one redshift measurement. Analysis of the radial profiles of galaxy surface density showed that most clusters were of the compact, regular type, indicative of advanced dynamical age. After taking into account selection effects due to k -corrections, the colour–magnitude relation for E and S0 galaxies, and accidental errors, and after careful treatment for foreground and background contamination, a trend of increasing fraction of blue galaxies with redshift was found for these compact clusters. It was suggested that this is evidence for a general depletion of gas in the Universe during recent epochs, and that exceptions to the trend indicate superimposed phenomena related to the particular evolution of each cluster.

Only one distant, rich cluster of the open or irregular type has been identified and studied so far, namely C11447+2619 (incorrectly called C11446+2619 in our previous work) at $z = 0.38$. Such systems are thought to be unrelaxed, and therefore to be of intermediate or young dynamical age. Nearby clusters of this type contain a galaxy-population mix intermediate between those of compact clusters and the field. C11447+2619 and the three nearby open systems were found to exhibit higher blue galaxy contents than the compact clusters, suggesting that characteristic population differences between cluster types obtain at all redshifts (at least to $z = 0.5$). In addition, the distribution of galaxy colours in C11447+2619 appears to be skewed more to the blue than is the case in any nearby population, even including the field sample. One is tempted, by continuity arguments, to speculate that the field population at high redshift will also be found to show signs of strong evolution.

Nearby open clusters contain elliptical, S0, and spiral galaxies roughly in the proportion E/S0/Sp = 1:2:3 (ref. 6). The frac-

tional blue galaxy content, f_B , as defined in ref. 3, for nearby open systems is $f_B \approx 0.15$. Thus, nearby open clusters have a ratio of spiral galaxy fraction, f_{Sp} , to blue galaxy fraction of $f_{Sp}/f_B \approx 3.3$. For C11447+2619, $f_B = 0.36 \pm 0.05$; if the ratio to spiral fraction is similar to that nearby, one would predict that all galaxies in the cluster must be spirals! The cluster is likely, therefore, to be an ideal testing ground for theories of morphological evolution between galaxy (Hubble) types, as well as an excellent site for the study of the photometric evolution of galaxies such as our own.

The photometric technique used to determine f_B relies on the statistical subtraction of foreground and background contamination across the field of the cluster. The fact that galaxies are not distributed randomly across the sky, but tend to clump in groups, clusters, clouds and superclusters, causes some uncertainty as to whether the decontamination has been correctly accomplished in any individual case and an accurate value for f_B determined. The only recourse is to obtain spectroscopy for as many galaxies as possible in the most interesting clusters identified photometrically. C11447+2619 is clearly one such cluster, and so we have tried to verify by spectroscopy its exceedingly large blue galaxy content.

The instrumental apparatus used for this work has been described in detail previously⁷. Briefly, it consists of a large format, low-noise charge-coupled device (CCD) detector in a low resolution, optically efficient spectrometer on the 4-m Mayall telescope at Kitt Peak. Masks having patterns of 2 arc s diameter apertures, configured to correspond to the locations of objects on the sky, are inserted at the entrance to the spectrometer, enabling simultaneous observation of many objects across a 5 arc min field of view. Round apertures have been used in preference to short slits because they permit work on larger numbers of galaxies in the rather crowded cluster field; they have the disadvantage of an increased susceptibility to the effects of atmospheric dispersion. For the present study, a resolution of 17 Å and a wavelength coverage of roughly 4,500–7,500 Å were used.

Two aperture plate masks were prepared for the C11447+2619 field, yielding spectra of 21 galaxies in the field of the cluster. None of the observations could be made near meridian passage, and no atmospheric dispersion compensation prisms were available. Therefore, the total integration was divided into 1-h segments, and these were distributed between the two aperture plates and over two nights of observation. Even so, the effects of atmospheric dispersion are evident among the data, and some data for outlying objects in some integration segments have been discarded. The resulting integration times for the 21 galaxies range from 2 to 5 h. Seeing during both nights was 2 arc s FWHM. Examples of the final spectra are shown in Fig. 1.

The results for the 21 galaxies are given in Table 1. The first 16 objects are from ref. 5, and include all blue galaxy candidates brighter than red magnitude 20.2, except one (no. 15). An additional five objects were observed, which lie outside the field having photometric data, but which seemed to be in the right magnitude range to be cluster members.

Of the total sample, two galaxies are seen clearly to be non-cluster members (nos 1 and a), and five (including no. 9) have spectra of too low a quality to yield a reliable redshift. Of the remaining 11 objects with photometry, seven are blue (according to the definition in ref. 3) and four are red. If we suppose that the uncertain objects among the first 16 are all unrelated to the

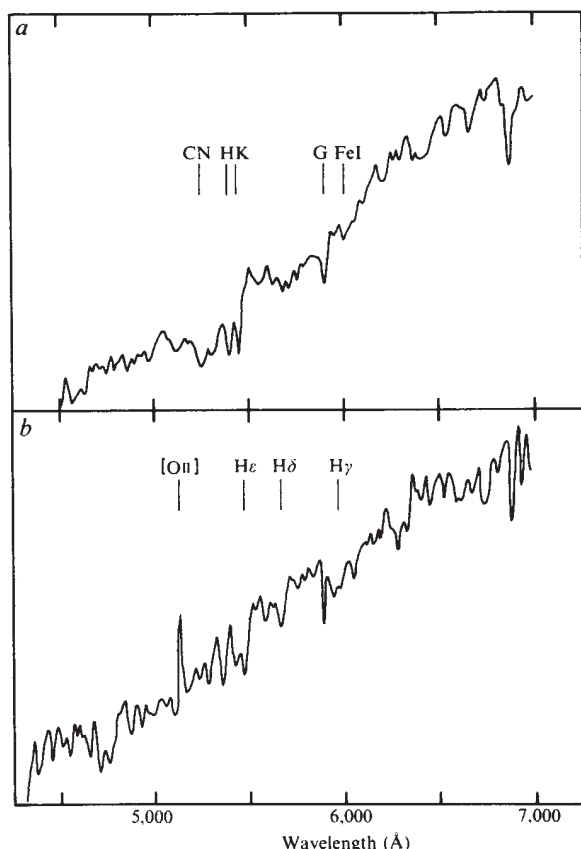


Fig. 1 Examples of spectra for galaxies in Cl1447 + 2619. Ordinate is flux per frequency interval in arbitrary units. Spectral resolution is 17 Å. *a*, Galaxy no. 2, a typical gE type spectrum with characteristic features marked. *b*, Galaxy no. 8, a typical E+A spectrum, which at lower signal-to-noise ratio would probably be called a dilute E spectrum. [O II] and several hydrogen lines are indicated, but gE features may also be seen by comparison with *a*.

cluster, then we have at least seven out of 12 blue galaxy candidates being cluster members. Our spectroscopy indicates that galaxies *b*, *c*, and *d* will be found to be blue photometrically, making at least 9 out of the 15 candidates certain or very probable members. Clearly, at least half of the candidate blue galaxies in the central regions of Cl1447 + 2619 are true cluster members. The rigorously defined, uniform sample of ref. 3 consists of 43 galaxies, 18 of which are blue. If we take the extreme case of all red galaxies being cluster members, we find that at least $18 \times \frac{9}{15} = 11$ out of 36 cluster members are blue, or $f_B \geq 31\%$. This minimum value can be compared with the photometrically derived blue galaxy fraction of 36% (ref. 3). Given the small sample of objects observed spectroscopically, the agreement is satisfactory. At the very least the spectroscopy may be said to confirm the absence of any significant error in the photometrically determined value.

Three high redshift clusters have now been examined spectroscopically, 3C295 (ref. 8), Cl0024 + 1654 (A. Dressler and J. E. Gunn, personal communication) and Cl1447 + 2619, and, in each case, the spectroscopic data support the reliability of the photometric procedure used in ref. 3 to identify the blue galaxy content. In addition, it is clear that most of the blue galaxies have spectra with features characteristic of stars and H II regions, rather than some more exotic process. Typically, the spectra may be described as 'dilute elliptical' galaxy spectra, often with [O II] $\lambda 3,727$ Å emission line equivalent widths in the range of integrated normal spiral galaxy spectra (although sometimes [O II] may be weaker than in normal galaxies of similar colour⁹). When the signal-to-noise ratio is high enough, the spectra generally seem to show A-type stellar features as well as elliptical

Table 1 Spectroscopic results for Cl1447 + 2619

No	(V-r)	z	Spectrum	Comments
1	B 1.05	0.231	gE	
2	1.47	0.373	gE	See Fig. 1
3	1.40	0.376	gE	Weak [O II]?
4	1.43	0.377	gE	Weak [O II]?
5	B 1.23	0.373	gE	
6	B 1.26	0.376	gE	
7	B 0.64	0.366	[O II], H β , [O III]	Not a Seyfert
8	B 1.02	0.378	E + A, [O II]	See Fig. 1
9	B 0.68	0.38::	Dilute E::	Data of low quality
10	B 0.84	0.38:	Dilute E:	Data a bit noisy
13	1.41	0.375	gE	
18	B 0.79	0.376	E + A, [O II]	
19	B 1.08	0.371	E + A	Or metal poor gE
21	B 0.61	—	—	Uncertain
32	B 0.72	—	—	Uncertain
33	B 0.61	—	—	Uncertain
<i>a</i>	—	0.42	gE	(+26, -135)
<i>b</i>	—	0.365	E + A	(-5, -134)
<i>c</i>	—	0.38:	[O II]:	(-38, -107)
<i>d</i>	—	—	—	(-45, -89)
<i>e</i>	—	0.368	gE	(-42, -82)

Object numbers in the first column are from ref. 5, except *a*-*e* which are identified by their offsets in arcseconds from object no. 2 as (Δ RA, Δ Dec) in the last column. The photometry listed in the second column is discussed in ref. 1. Those objects qualifying as blue according to the definition in ref. 3 are indicated with a B. The spectra here are characterized as follows: gE, giant elliptical galaxy spectrum similar to that shown in Fig. 1*a*; dilute E, spectral features similar to gE, but weaker, and with a bluer continuum; E + A, apparent superposition of gE and A-type stellar spectra, or dilute E with strong hydrogen absorption lines; [O II], H β , [O III], emission lines at λ 3,727, 4,863, and 4,959 + 5,007 Å, respectively. The observed equivalent widths for [O II] in galaxies 3, 4, 7, 8, 18 and *c* are 8-, 15-, 38, 19, 29, and 28 Å, respectively.

galaxy characteristics; the blue galaxy spectrum in Fig. 1 is typical of such spectra in our experience (see also ref. 8).

Evidently then, the vast majority of these objects are unexpectedly blue because of enhanced star formation somewhere in the galaxy. Imagery at roughly 10 times the best available resolution will be required to delineate just what is happening in the galaxies in Cl1447 + 2619 and other high redshift clusters. In the absence of such imagery, one can ask whether observations of local group galaxies might not uncover evidence for a period of enhanced star formation, several Gyr and more ago, which in turn might be used to shed light on the nature and development of the phenomenon. Such evidence may, in fact, already have been reported, with the Large Magellanic Cloud¹⁰⁻¹² and Carina dwarf galaxy¹³ found to have main sequence luminosity functions strongly suggesting accelerated star formation 3-5 Gyr and 6-9 Gyr ago, respectively. The presence of carbon stars in other local group dwarfs¹⁴ may also indicate episodes of strong star forming activity at intermediate epochs.

Whether the history of these dwarf galaxies has a direct bearing on the evolution seen among cluster galaxies is an open question. The only nearby, intrinsically-bright galaxy for which an analysis can be made is our own. Here the matter is unclear, complicated as it is by our being inside the system. Suffice it to say that a period of moderately enhanced activity in the solar neighbourhood at the epochs under discussion is not ruled out by available data, and the situation at other galactic radii is completely unknown.

Much has been made of the consistency of available evidence with a constant or exponentially declining rate of star formation in galaxies beginning a Hubble time ago¹⁵⁻¹⁷. However, in view of the above discussion we should remark that we know of no evidence clearly inconsistent with the hypothesis that most galaxies experienced their maximum star forming activity at intermediate epochs.

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1. Butcher, H. & Oemler, A. Jr *Astrophys. J.* **219**, 18–30 (1978).
2. Butcher, H. & Oemler, A. Jr *Astrophys. J.* **226**, 559–565 (1978).
3. Butcher, H. & Oemler, A. Jr *Astrophys. J.* (in the press).
4. Couch, W. J. thesis, Australian National Univ. (1981).
5. Butcher, H., Oemler, A. Jr & Wells, D. C. *Astrophys. J. Suppl.* **52**, 183–228 (1983).
6. Oemler, A. Jr *Astrophys. J.* **194**, 1–19 (1974).
7. Butcher, H. *Proc. S.P.I.E.* **331**, 296–300 (1982).
8. Dressler, A. & Gunn, J. E. *Astrophys. J.* **270**, 7–19 (1983).
9. Dressler, A. & Gunn, J. E. *Astrophys. J.* **263**, 533–545 (1982).
10. Butcher, H. *Astrophys. J.* **216**, 372–380 (1977).
11. Stryker, L. L. *IAU Symp. No. 108*, 79–80 (1983).
12. Linde, P., Ardeberg, A., Lindgren, H. & Lyngå, G. *IAU Symp. No. 108*, 99–100 (1983).
13. Mould, J. & Aaronson, M. *Astrophys. J.* **273**, 530–538 (1983).
14. Aaronson, M., Olszewski, E. W. & Hodge, P. W. *Astrophys. J.* **267**, 271–279 (1983).
15. Searle, L., Sargent, W. & Bagnuolo, W. *Astrophys. J.* **179**, 427–438 (1973).
16. Larson, R. & Tinsley, B. *Astrophys. J.* **219**, 46–59 (1978).
17. Kennicutt, R. *Astrophys. J.* **272**, 54–67 (1983).

A three-dimensional model of the fluid dynamics of radio-trail sources

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Extragalactic radio sources display a wide range of complex structures. The weaker sources have twin jets emerging from the nucleus of the parent galaxy, which bend and twist as they interact with the intergalactic medium (IGM); extreme examples are the radio-trail sources, such as NGC1265 (ref. 1) and 3C129 (ref. 2). More powerful sources typically show double structures with lobes and hotspots, but even these (such as Cygnus A (R. A. Perley, personal communication)) contain curved jets. Although our theoretical understanding of sources in terms of twin plasma-jets has advanced, numerical simulations^{3,4} have concentrated on axisymmetric models, whereas the investigation of radio-trail and complex sources demands three-dimensional simulations, despite the obvious computational difficulties. It is now generally accepted that radio-trail galaxies are produced by the motion of active galactic nuclei through the IGM in clusters of galaxies⁵. Models were initially proposed in which the ejected material consisted of independent blobs or plasmoids^{6–8}, but most evidence^{9,10} now supports ejection in a quasi-continuous jet^{9,11}. Here we adopt the jet model and study the formation of twin-tail sources by the motion of the active nucleus through intra-cluster gas, using three dimensional fluid-dynamical simulations of a supersonic jet in a cross-wind.

We integrated the non-relativistic equations of ideal compressible flow by an eulerian finite-difference method assuming magnetic forces to be dynamically unimportant. Our hydrodynamic code was based on the 'fluid-in-cell' method of Gentry *et al.*¹² modified to use the 'flux-corrected transport' algorithm of Book *et al.*¹³. This version substantially reduces numerical mass-diffusion at the contact discontinuity between shocked IGM and shocked ex-jet material, a well-documented difficulty with eulerian codes¹⁴. The multi-dimensional equations were solved using the time-splitting technique of Strang¹⁵. The simulations were performed on the Starlink VAX 11/780 at Cambridge.

The calculations were carried out on an (x, y, z) grid of $144 \times 72 \times 8 = 82,944$ cells. Because of limitations in computing time and storage we stretched the cells in two directions to provide a domain $144 \times 216 \times 16$ (so that the computing cells are rectangular parallelepipeds with sides $1 \times 3 \times 2$), and later rezoned the problem onto a domain $288 \times 216 \times 16$ to allow the calculation to proceed. We simulated the motion of an active nucleus through a cluster gas, in the rest frame of the nucleus, by giving all cells initial values corresponding to a constant density, pressure and velocity. The direction of motion of the galaxy was taken to be the x -axis and the initial jet direction along the

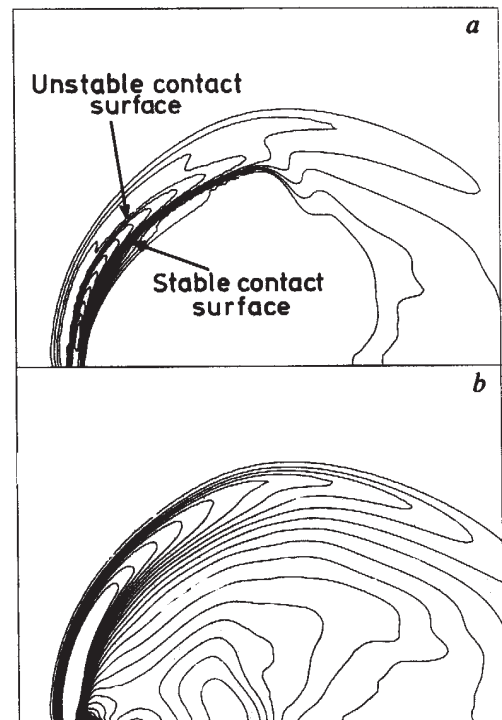


Fig. 1 The distribution of: *a*, density; *b*, pressure in the $z=0$ plane of a slow heavy jet at $t=68.7$. The contours are logarithmic, contouring ratios 1.15:1.

y -axis. For this particular case there are two mirror-symmetry planes; as well as the $z=0$ plane containing the centre line of the jet, the flow was taken to be symmetrical about the $y=0$ plane, which separates the two jets. Three types of boundary conditions were used at the edges of the computing grid: (1) input, where jet parameters were specified at the beginning of each time step; (2) continuative, where fluid was allowed to flow into or out of the computing mesh; (3) reflective, where the normal component of velocity was set to zero.

Input conditions were specified where the jet entered the computing grid. Continuative conditions were used elsewhere except in the $y=0$ and $z=0$ planes. Although we have modelled the flow pattern with two mirror planes, we do not expect that, in practice, the flow will be strictly symmetrical downwind of the jet; Kelvin–Helmholtz instabilities upwind of the jet may be convected downwind preventing strict symmetry there. Thermal pressure balance between the unshocked jet and unshocked IGM was assumed. The jet parameters were chosen to keep the ratio of momentum fluxes constant between simulations as in Table 1. Assuming that the radius of curvature of the jet is given simply by

$$R = r_j \rho_j v_j^2 / \rho_m v_m^2$$

where ρ_m and v_m are the density and speed of the IGM, and ρ_j , v_j and r_j are the density, speed and radius of the jet on entry to the computing grid, this condition would make the path of the jet the same in each simulation. The jet was injected with a staircase-circular cross-section and 'radius' four units corresponding to four zones in the x -direction and two zones in the z -direction. The structure near the entry point, in particular the oblique entry-shock mentioned below, was studied in greater detail using a jet of 'radius' eight zones.

The distribution of density and pressure in the $z=0$ plane of a slow, heavy jet corresponding to case (a) in Table 1 is shown in Fig. 1. An interesting feature is that the outer contact surface is badly smeared out, despite the measures taken in the code to combat diffusion¹³. By way of comparison the inner contact surface may be clearly seen. Although our code is incapable of modelling accurately the growth of small-scale instabilities, simple arguments suggest that the centripetal acceleration

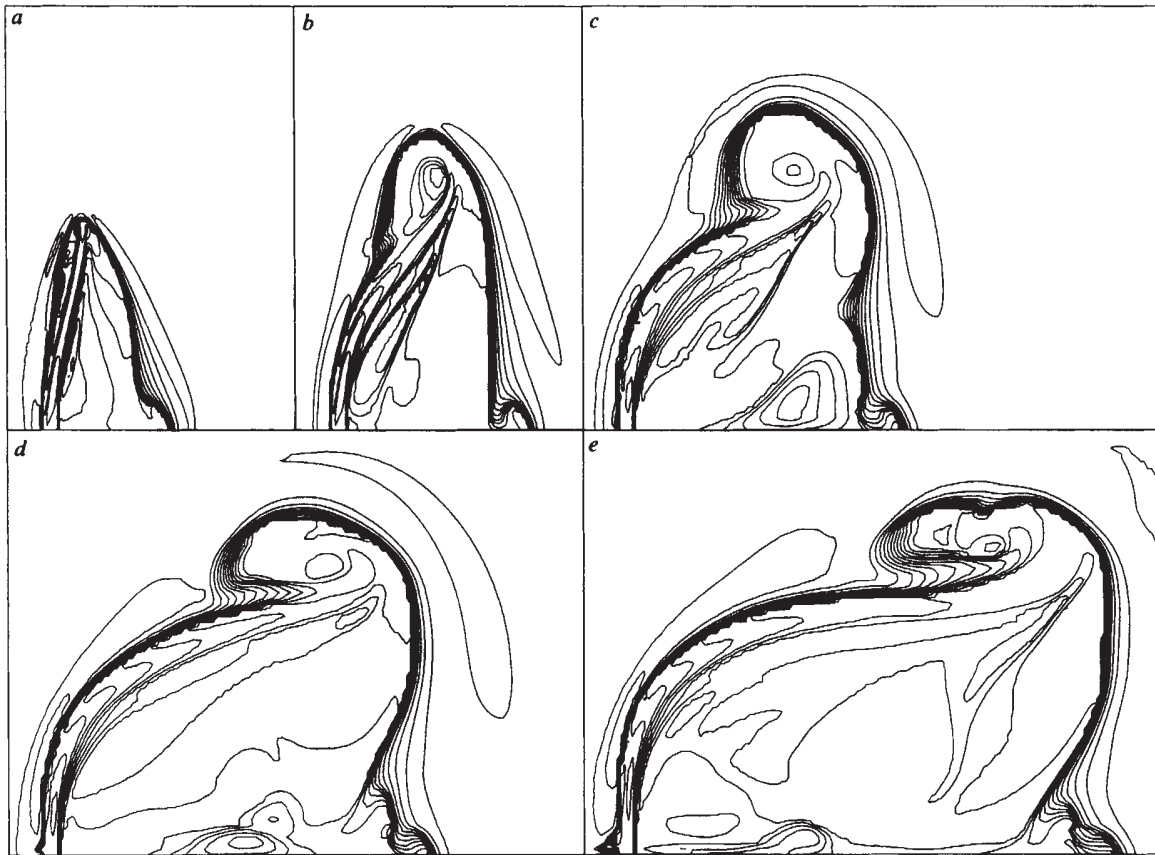


Fig. 2 The distribution of density in the $z=0$ plane of a fast, light jet at various epochs. The density contours are logarithmic, contouring ratios 1.5:1. *a*, $t=11.2$; *b*, $t=20.9$; *c*, $t=34.3$; *d*, $t=49.4$; *e*, $t=65.3$.

required to bend a heavy jet renders it Rayleigh–Taylor unstable along its outer edge. Whilst the growth of these instabilities is complicated by the shear across the contact surface, this possible instability may pose problems for models involving heavy jets. We find that light jets overcome the complementary problem of disruption by a Rayleigh–Taylor instability on their inner surface by inflating a cocoon of shocked ex-jet products.

Figure 2 shows the distribution of density in the $z=0$ plane of a light, fast jet (Table 1, case (b)) at various epochs after switching on the jet, while Fig. 3 shows the distribution of pressure and velocity vectors corresponding to the density distributions in Fig. 2*b,c*. Some initial deflection is caused by an oblique shock as the jet enters the computing mesh, but thereafter bending is caused by the pressure gradients across the jet. The density contours in Fig. 2 show that this bending is accompanied by an expansion wave. Two effects would seem to be responsible: first, the stagnation pressure reaches a maximum at $z=0$, producing an expansion in the z -direction; second, in this particular case where the initial jet direction is perpendicular to the direction of motion of the galaxy, the stagnation pressure decreases monotonically along the direction of jet propagation.

Although the density of the jet drops by an order of magnitude in bending through 90° , Fig. 3*a, c* shows that the speed of the

flow actually decreases along the jet. There is, therefore, considerable departure from steady isentropic quasi-one-dimensional flow for which we would expect $d\rho/\rho = -M^2 dv/v$. Four factors may be responsible.

- (1) A transient phenomenon related to the hook-like structure described below indicates that the flow may not yet be steady.
- (2) The quasi-one-dimensional approximation may break down both because the cross-section of the jet increases too rapidly and because the flow parameters vary across the jet.
- (3) The bending is not isentropic because of a combination of physical and numerical effects; dissipation may occur due to viscosity, numerical mass diffusion and thermal conduction. In particular, we find the temperature increases along the direction of jet propagation. Higher resolution studies will help to elucidate this detail.
- (4) The density contours and velocity vectors suggest that some entrainment is occurring along the outer edge of the jet.

The cross-section of the jet is deformed into a kidney-shape by the pressure gradients across it, in agreement with wind-tunnel experiments¹⁶. The cocoon, which is inflated downwind of the jet, is much larger than in comparable axisymmetrical simulations, due to the motion of the galaxy, and contains material which is actually lighter than the jet, in particular, a region of hot, light gas downwind of the jet. For comparison, we find that in axisymmetrical simulations the jet is completely surrounded by a narrow trench of hot, light gas, separating it from the axisymmetrical backflow of shocked ex-jet material. We interpret this hot, light gas as arising from dissipation (possibly slightly over-efficient in our numerical code) caused by short wavelength Kelvin–Helmholtz instabilities between the jet and its backflow.

Near the point of entry onto the computing grid, the jet adapts to the ram pressure of the cross-wind by means of an oblique shock. While this is not directly relevant to radio sources, jets may respond to inhomogeneities in the external medium by an oblique shock of this sort, for example, in the knots of 3C83.1B

Table 1 Jet parameters

	ρ_j	P_j	M_j	v_j	ρ_m	P_m	v_m
(a)	10.0	1.0	10.0	4.082	1.0	1.0	3.0
(b)	0.1	1.0	10.0	40.82	1.0	1.0	3.0
(c)	0.1	1.0	5.0	20.41	1.0	1.0	1.5
(d)	0.1	1.0	100.0	408.2	1.0	1.0	30.0

P_j and P_m are the thermal pressures in the unshocked jet and unshocked IGM. M_j is the Mach number of the jet on entry to the computing grid.

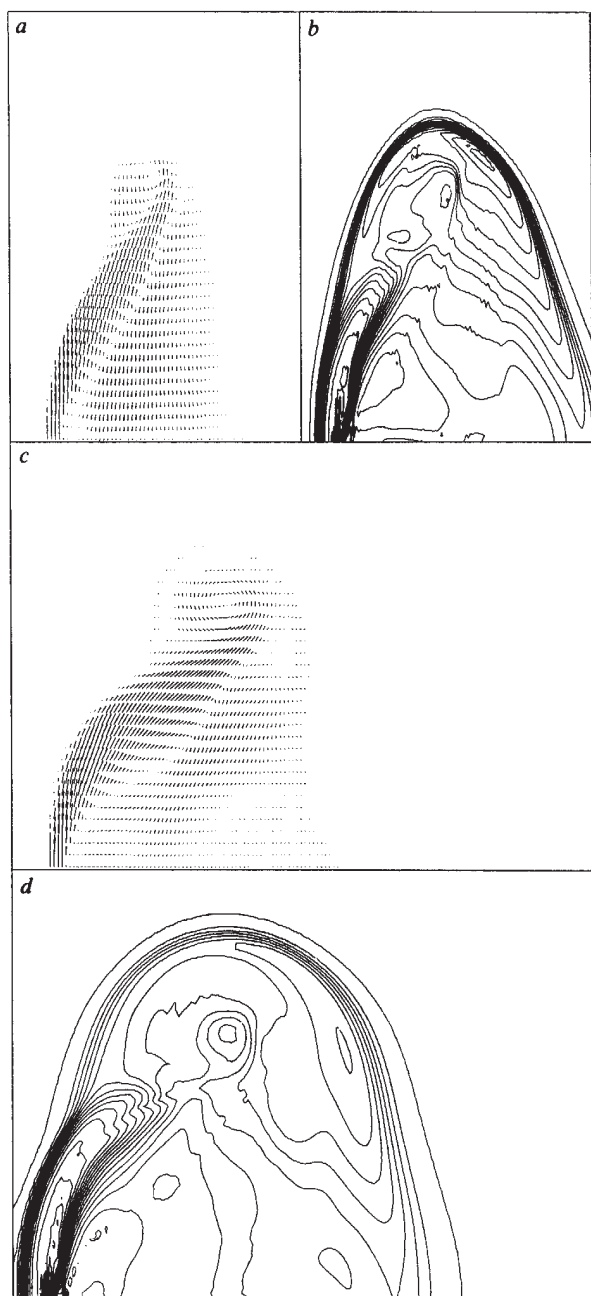


Fig. 3 Velocity vectors and the distribution of pressure in the $z=0$ plane of a light jet at $t=20.9$, *a* and *b*, and at $t=34.3$, *c* and *d*. The pressure contours are logarithmic, contouring ratios 1.15:1. Velocity vectors are shown at every other grid point.

(ref. 10). The stagnation pressure of the bending jet is lower than the hotspot pressure in comparable axisymmetrical simulations by a factor R/r_j . This opens the possibility, therefore, that Fanaroff and Riley (FR) class I¹⁷ sources have low luminosities compared with class II sources, partly because they are distorted by their environment. The jet shock also decreases in strength (Fig. 3*b, d*) in agreement with the observation that radio-trail sources do not have hotspots. In the simulations, three effects seem to be responsible for this decrease in strength: first, the decrease in density and speed described above reduces the ram pressure in the jet; second, the Mach number of the jet is reduced by entrainment of shocked IGM at the vortex; finally, the ambient IGM is retreating from the jet once it begins to bend, further reducing the effective Mach number.

The sudden expansion of some twin-tail sources after they have been bent through large angles may indicate the onset of

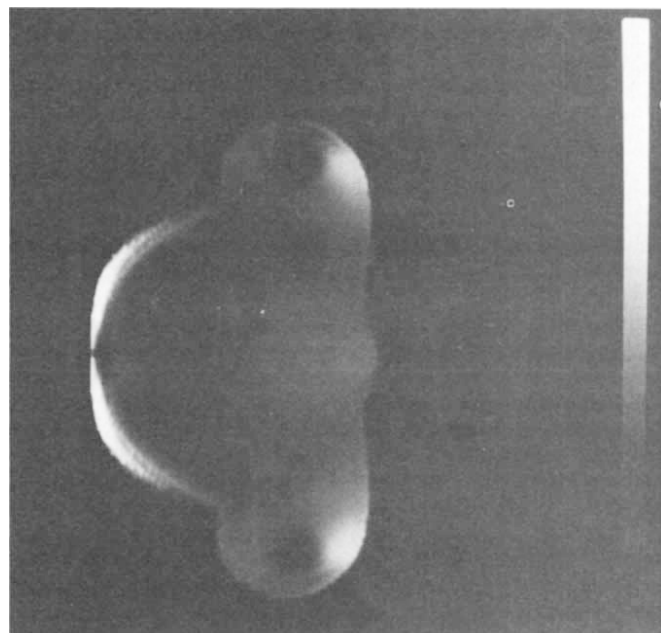


Fig. 4 The simulated radio emission at $t=34.3$.

turbulence due to this reduced Mach number (beyond the point that we have so far been able to follow by calculation). A possible example of a source with the morphology shown in Fig. 2 is IC708, whose structure has been previously attributed to gravitational encounters¹⁸.

In addition to the effects reported in axisymmetrical simulations³, the cocoon in radio-trail sources is dynamically important to the evolution of the jet because in the later epochs of Fig. 2, the jet interacts with the downwind wall of its cocoon. This results simply from the supersonic character of the flow. To bend the jet, pressure differences must be established across it, for which several sound-crossing times will be necessary. The bending of the supersonic jet therefore lags behind its advance as may be seen in the early epochs of Fig. 2. Further bending of the jet causes it to collide obliquely with the downwind wall of its cocoon, bending the tip of the jet upwind in a hook-like feature and producing a low-pressure vortex upwind of the jet. We investigated the effect of varying the Mach number of the jet by computing the flow patterns expected in Mach 100 and Mach 5 jets (Table 1, cases *c* and *d*). The results show that the hook-like feature is more prominent in highly supersonic jets. A possible explanation is that the distance the jet will advance, in the time before the pressure gradient required to bend the jet is established, is greatest for the most supersonic jets. This will depend on some average of the Mach number over the jet cross-section, which is not constant but varies due to the pressure gradient. At its downwind edge $M = M_j$, the entry Mach number of the jet, while at its upwind edge $M = (R/r_j)^{1/2}$ independent of M_j .

A comparison of the results of these numerical simulations with maps of radio-trail sources suggests that most of the radio emission probably only occurs in shocked jet material and not in the shocked IGM behind the bow-shock. Figure 3 shows that the cross-wind merely displaces the bow shock downwind rather than changing its shape appreciably, whereas radio-trail sources commonly display concave features, for example, NGC1265 (ref. 1) and 3C129 (ref. 2). For this reason Fig. 4, which shows the simulated radio emission calculated by integrating the thermal pressure in a line-of-sight through the source, shows only the radio emission expected from inside the contact surface. The simple prescription for calculating radio emission assumes that the energy in relativistic particles and magnetic fields, which are responsible for the radio emission, may be approximated by a fixed fraction of the thermal pressure provided the necessary

seed populations are present. It is possible that the jets, which seem probably to be light and fast, may be jets of electron-positron plasma containing an enhanced seed population of mildly relativistic particles.

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1. Miley, G. K., Wellington, K. J. & van der Laan, H. *Astr. Astrophys.* **38**, 381–390 (1975).
2. Downes, A. J. B. *Mon. Not. R. astr. Soc.* **190**, 261–268 (1980).
3. Norman, M. L., Smarr, L., Winkler, K.-H.A. & Smith, M. D. *Astr. Astrophys.* **113**, 285–302 (1982).
4. Wilson, M. J. & Scheuer, P. A. G. *Mon. Not. R. astr. Soc.* **205**, 449–463 (1983).
5. Miley, G. K., Perola, G. C., van der Kruit, P. C. & van der Laan, H. *Nature* **237**, 269–272 (1972).
6. Cowie, L. L. & McKee, C. F. *Astr. Astrophys.* **43**, 337–343 (1975).
7. Jaffe, W. J. & Perola, G. C. *Astr. Astrophys.* **26**, 423–435 (1973).
8. Pacholczyk, A. G. & Scott, J. S. *Astrophys. J.* **203**, 313–322 (1976).
9. Jones, T. W. & Owen, F. N. *Astrophys. J.* **234**, 818–824 (1979).
10. Owen, F. N., Burns, J. O. & Rudnick, L. *Astrophys. J. Lett.* **226**, L119–L123 (1978).
11. Begelman, M. C., Rees, M. J. & Blandford, R. D. *Nature* **279**, 770–773 (1979).
12. Gentry, R. A., Martin, R. E. & Daly, B. J. *J. computat. Phys.* **1**, 87–118 (1966).
13. Rook, D. L., Boris, J. P. & Hain, K. J. *computat. Phys.* **18**, 248–283 (1975).
14. Sod, G. A. *J. computat. Phys.* **27**, 1–31 (1978).
15. Strang, G. *SIAM J. numer. Analysis* **5**, 507–517 (1968).
16. Kamotani, Y. & Greber, I. *Am. Inst. Aeronaut. Astronaut. J.* **10**, 1425–1429 (1972).
17. Fanaroff, B. L. & Riley, J. M. *Mon. Not. R. astr. Soc.* **167**, 31P–35P (1974).
18. Vallee, J. P., Bridle, A. H. & Wilson, A. S. *Astrophys. J.* **250**, 66–78 (1981).

Kinetic energy spectrum of large- and mesoscale atmospheric processes

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Considerable attention has recently been focused on the distribution of atmospheric kinetic energy over the range of scales often referred to as the mesoscale. Despite the importance of the mesoscale range of wavelengths (10^1 – 10^3 km) for dynamical analyses and for numerical weather prediction, there have been few measurements of the kinetic energy spectrum over this range. Here, we report the results of a new determination of the kinetic energy spectrum of winds near the tropopause level for the wavelength range 2.6 – 10^4 km. This spectrum is based on wind measurements made on board airliners in routine commercial service. We find that the spectrum obeys a $-5/3$ power law dependence on wavenumber over the wavelength range ~ 2.6 – 300 km and a -3 power law over the range $1,000$ – $3,000$ km. The spectral magnitudes of the zonal and meridional winds are very similar, especially over the $-5/3$ region.

Mesoscale processes, which operate on horizontal scales from 1 km or so to several hundreds of kilometres, bridge the region in size between the synoptic- and planetary-scale motions resolved on routine weather maps and the microscale motions where viscous dissipation acts to reduce the atmosphere's total kinetic energy. Among meteorologists, it has been widely believed that the kinetic energy spectrum should have reduced magnitude at the mesoscale¹; there should be a mesoscale gap in the spectrum. It is sometimes argued that a mesoscale gap would favour the predictability of atmospheric motions at the larger scales. However, there is increasing evidence that mesoscale processes contain significant amounts of energy and can act as energy sources for the large-scale motions, at least in the upper troposphere². One study³ concludes that "the parameterization formulae commonly used in the numerical models of the atmospheric large-scale flow for the representation of friction due to unresolved horizontal eddies seem to have nothing to do with the corresponding observed force".

Before this problem of parameterization can be solved, it is necessary first to observe and to understand the mesoscale portion of the kinetic energy spectrum. An acute problem in studying mesoscale motions in the upper troposphere and lower stratosphere has been the lack of suitable spatial data. At scales

<100 km or so, data from research aircraft have been used^{4–6}, and at scales of >1,000 km or so, radiosonde data have been used^{7–9}. Between these two extremes, the only appropriate data source available seems to be from measurements made on commercial aircraft. If the frozen turbulence hypothesis is valid at mesoscales, then the Taylor transformation could be applied to convert single-station frequency spectra into wavenumber spectra. There have been reservations about using the Taylor transformation at relatively long periods although recent evidence suggests it may be valid¹⁰. In any case, the most desirable data are direct spatial measurements through the mesoscale range.

Aircraft data are, in fact, available over the wavelength range 10^0 – 10^4 km. The wind data used here were collected as part of the Global Atmospheric Sampling Program (GASP)¹¹. The data collection phase of GASP was conducted during 1975–79, with meteorological and trace constituent data automatically recorded with instruments placed on board Boeing 747 airliners in routine commercial service. Wind data were taken from the onboard computer, which was linked to the inertial navigation system, and have a random error of 5% of the reported value. There are 6,945 flights in the GASP data set, with over 0.6×10^6 observations. All GASP data are archived at the National Climatic Center, Asheville, North Carolina.

Figure 1 shows the spectra of zonal and meridional winds over the range of wavelengths 2.6–10,000 km. GASP data were recorded under two modes, and Fig. 1 was prepared in two parts. On most flights the data were recorded at 5-min intervals (75-km intervals at a nominal ground speed of 250 m s^{-1}) at all times during flight above 6 km; about 80% of all data fall in the altitude range 9–13 km. For the portion of the spectra in Fig. 1 between 150 and 10^4 km, the data were interpolated to 75-km intervals for the 311 flights which were more than 10^4 km long and which had an average orientation of east–west. All of these flights were between New York and Japan, New York and cities on the Persian Gulf, or California and Japan or Hong Kong, and the average latitude of these data is 50° N . There were very few missing data; if two consecutive points on a flight were missing, the data for that flight were not used. The mean and a linear trend were removed from the interpolated data, and then a Fast Fourier Transform was applied. The sums of the squares of the Fourier coefficients at each wavelength were averaged and the means are shown in Fig. 1 (solid circles).

On 97 flights, the GASP system was set to record data each 4 s (nominally 1 km), rather than 5 min. These flights were divided into segments which were 150 km long. If more than five data points were missing during any minute, that segment was not used. The data were interpolated to 1.3-km intervals, the mean and linear trend removed, the spectral analysis applied, and the spectral estimates at 2.6–150 km wavelength were averaged and plotted in Fig. 1 (open circles). There were 1,492 suitable segments; the average latitude of these data is 29° N . At wavelengths <5 km, three points were averaged and plotted as one; no other smoothing was applied.

Standard deviations of the spectral estimates are approximately the same size as the mean values at all wavelengths. The error bars plotted in Fig. 1 approximate the 95% confidence intervals at all wavelengths. They extend above and below the mean value by an amount double the standard deviation divided by the square root of the number of flights. Straight lines with slopes -3 and $-5/3$ intersect the same coordinates for both the zonal and meridional curves, for comparison.

There is no evidence of a broad mesoscale gap in the spectra in Fig. 1. Indeed, the spectra are remarkably continuous over the entire wavelength domain portrayed. To illustrate the degree of continuity and the blending of the two sections of the spectra, data from the 97 high-data-density flights were analysed over long segments and the results are plotted in Fig. 1 (crosses) across the wavelength range 25–1,500 km. They represent the average spectral amplitudes of the 39 flight segments which had data spaced at 1.3-km intervals with no more than five missing observations during any minute and which were at least 1,500 km

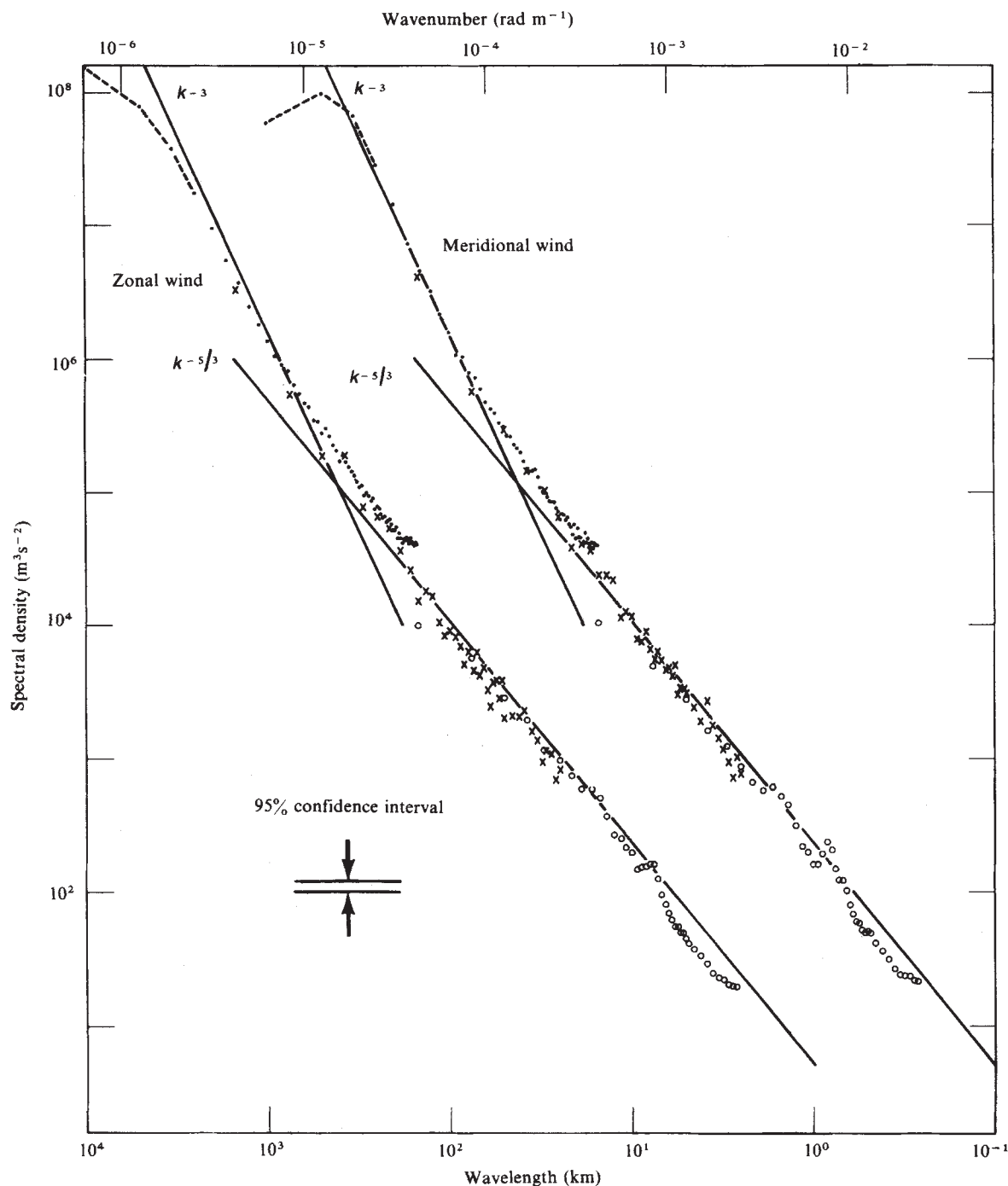


Fig. 1 Kinetic energy spectrum near the tropopause from GASP aircraft data. The spectrum for meridional winds is shifted one decade to the right. Spectral averages based on long- (●), short- (○) and intermediate- (×) scale data are shown.

long. The average latitude of these 39 segments is about 30° N. The crosses blend smoothly with the short-wavelength sections of the spectra; this is expected as they are based on a subset of the same data. Also, the crosses follow the long-wavelength sections of the spectra fairly closely, but have a slightly reduced amplitude (~80%) over the wavelength range 150–1,500 km. This reduced amplitude may be due to sampling fluctuations, or it may be explained by the lower latitude of the data used for the crosses; that is 30° N versus 50° N. Results are given in Fig. 1 as a function of wavelength and the number of waves per metre; rather than as a function of the number of waves around a circle of latitude (the zonal wavenumber). Large-scale atmospheric disturbances often extend over a significant range of latitude, and their spectra at different latitudes are sometimes normalized by referring them to a common zonal wavenumber¹². In this case, the appropriate normalizing factor for the crosses is the ratio of the cosine at 30° N to that at 50° N, which is 1.35.

This normalizing factor would make the two sets of spectral estimates agree within 10%.

The apparent high energy at the shortest few wavelengths of each section of the spectra may be due to aliasing by unresolved scales. The apparent low energies in the short-wavelength sections of the spectra at 150 km wavelength are due to the filtering effects of the linear trends removed. The spectral estimate at 10⁴ km wavelength is only slightly reduced due to trend removal, because the power there increased by less than a factor of two when the spectra were recomputed without any trend removed. The magnitudes of the spectra in Fig. 1 agree within a factor of two with past estimates from radiosonde data^{7–9}, and with research aircraft data¹³ and our own preliminary analysis over the wavelength range 150–2,400 km (ref. 14), both based on a relatively very small sample of flights.

At synoptic-scale wavelengths, from about 1,000 to 3,000 km, the observed -3 power law dependence on wavenumber is

considered evidence of an enstrophy cascade from low to high wavenumbers, and this process has been described as geostrophic turbulence¹⁵. At scales below about 500 km the $-\frac{5}{3}$ power law dependence may be interpreted as a reverse energy cascade from high to low wavenumbers in quasi-two-dimensional turbulence^{16–18}, or it may be interpreted as a spectrum of internal gravity waves with direct energy cascade to high wavenumbers, as oceanic spectra are interpreted^{19,20}. Clearly, it is important to understand whether this part of the spectrum is determined by turbulent or by wave dynamic processes. This issue cannot be resolved here, but now that the basic spectrum has been described over a wide range of wavelengths, it provides a framework in which to view small portions of the spectrum where data are more plentiful. We are preparing climatological studies of the spectra of wind and temperature for the wavelength ranges 2–150 km and 150–2,400 km from the GASP data. These studies will further our understanding of the dynamical origins of the atmospheric kinetic energy spectrum.

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1. Fiedler, F. & Panofsky, H. A. *Bull. Am. met. Soc.* **51**, 1114–1119 (1970).
2. Vincent, D. G. & Schlatter, T. W. *Tellus* **31**, 493–504 (1979).
3. Holopainen, E. & Nurmi, P. *Tellus* **32**, 124–130 (1980).
4. Pinus, N. Z. *Izv. Atmos. Ocean. Phys.* **13**, 808–814 (1977).
5. Pinus, N. Z. *Izv. Atmos. Ocean. Phys.* **15**, 93–97 (1979).
6. Kao, S. K. & Woods, H. D. *J. atmos. Sci.* **21**, 513–519 (1964).
7. Chen, T. C. & Wiin-Nielsen, A. *Tellus* **30**, 313–322 (1978).
8. Kao, S. K. & Wendell, L. L. *J. atmos. Sci.* **27**, 359–375 (1970).
9. Boer, G. J. & Shepherd, T. G. *J. atmos. Sci.* **40**, 164–184 (1983).
10. Brown, P. S. & Robinson, G. D. *J. atmos. Sci.* **36**, 270–286 (1979).
11. Perkins, P. & Gustafsson, U. R. NASA-TMX-71790 (NASA, Washington, DC, 1975).
12. Tang, C. M. & Orszag, S. A. *J. Fluid Mech.* **87**, 305–319 (1978).
13. Lilly, D. K. & Petersen, E. *Tellus* **35A**, 379–382 (1983).
14. Nastrom, G. D. & Gage, K. S. *Tellus* **35A**, 383–388 (1983).
15. Charney, J. G. *J. atmos. Sci.* **28**, 1087–1095 (1971).
16. Kraichnan, R. H. *Phys. Fluids* **10**, 1417–1423 (1967).
17. Gage, K. S. *J. atmos. Sci.* **36**, 1950–1954 (1979).
18. Lilly, D. K. *J. atmos. Sci.* **40**, 749–761 (1983).
19. Dewan, E. M. *Science* **204**, 832–835 (1979).
20. VanZandt, T. E. *Geophys. Res. Lett.* **9**, 575–578 (1982).

Volcanic, CO₂ and solar forcing of Northern and Southern Hemisphere surface air temperatures

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Seasonally-resolved energy balance models having air–surface, land–ocean and Northern and Southern Hemisphere resolution are used to elucidate the possible relative importance of several external factors on the climate of the past century. The model used here allows a direct comparison of observed and simulated temperatures from the same physical domains—over land and sea separately in each hemisphere. The availability of independent temperature records in two hemispheres significantly increases the number of independent degrees of freedom in the data available to ‘verify’ the simulations. Independent volcanic effects are found in both hemispheres, while similar responses for the more globally distributed CO₂ and tentatively identified solar forcings are found in the two hemispheres. The empirically derived CO₂ equilibrium doubling response for air surface temperature is $1.6 \pm 0.3^\circ\text{C}$, although the statistical significance of this result is uncertain.

A previous study¹ used a yearly average model² of globally-averaged climate (essentially an ocean mixed-layer model with no surface–air distinction) for simulation of a hemispheric scale temperature, which was compared with observations of surface air temperatures over land. Hansen *et al.*³ used a yearly and globally averaged radiative–convective model (with vertical res-

olution) to simulate the climate of the past century, again comparing with a largely land-derived set of surface air temperature records. Here we have used a climate model based on energy balance and exchange between 10 ‘boxes’: air over land, air over ocean, land surface, mixed layer and deep ocean—all resolved hemispherically with temperature as the dependent variable. Details of the model may be found elsewhere^{4,5}; we merely enumerate here for convenience some of the model attributes. All primary parameters such as mixed-layer depth, cloud fractions, albedos, insolation and interhemispheric energy exchange are functions of season based on observations⁶ where available and estimation from physical principles elsewhere. Where large uncertainties exist in model parameters, (for example, coefficient parameterizations between various boxes) these have been adjusted in a least-squares sense (as in ref. 1) to best fit the mean observed seasonal temperature variations^{7,8}. (Here we use the same value of seasonal interhemispheric heat exchange in every year, while the original model⁵ used inter-hemispheric temperature difference to parameterize heat exchange.) The model uses 1-day time steps, 1-month averages for seasonal calibrations and yearly means for the climate simulations. The correlation coefficients for the seasonal fits (air over land, air over ocean, mixed-layer temperature for two hemispheres) average 0.99, implying that the model has the correct form and phase (upper limit on phase lag between model and observations for $r = 0.99$ is 8.1 days) for the seasonal variations. Note that the correlation coefficient of two curves does not depend on relative amplitudes or zero point differences, although the model performs adequately for these also⁵.

We have adopted area-weighted, surface air over land, hemispheric average temperatures as our modulus of observed climatic change. This provides the most direct and reliable empirical measure of climatic change available and is convenient to match to the model output. The problem of earlier studies—not having a good hemispheric observational record due to lack of land and sea coverage—no longer applies, as we can now match model and observations separately over land and sea. Even so, the temperature records must still be taken as only an approximation to reality⁹. The hemispheric means used (land only) are shown in Fig. 1a, b taken over latitudes 5–85° for the Northern and 0–45° for the Southern Hemisphere. The caveats given by Jones *et al.*¹⁰ concerning the Northern Hemisphere data set apply even more strongly to the Southern Hemisphere record over 1881–1980 derived from a preliminary compilation by the same authors. The Southern Hemisphere mean represents only a small fractional area and the early part of the record is based only on about six gridpoints, thus the early part of the Southern Hemisphere record is of particularly marginal quality. Despite these problems, we feel that the methods (if not the results) we use deserve further consideration.

We consider the effects of the three most commonly cited external causes of climatic change. CO₂ is important for the effects it may have had in causing or modifying climate on ice-age time scales^{11,12} and, of course, for its possible twentieth century influence. Solar forcing is universally recognized as potentially important if the Sun is variable at a level $>0.1\%$ extending over several years. We do not yet have direct evidence of long-term solar variations and so treat this as the most hypothetical of our three forcings. At the least, the solar forcing is reasonable, and is useful as a test of how sensitive the empirically derived CO₂ response is to its inclusion. The only known component of solar variability, sunspot blocking, leads to negligible hemispheric-scale surface temperature variations¹³. Volcanic perturbations resulting from stratospheric aerosol injections are widely recognized as the only external forcing whose importance has been verified^{14–17}.

The concentration of atmospheric CO₂ has been accurately measured¹⁸ as having increased from 315 p.p.m. in 1959 to >340 p.p.m. in 1983. This 8% increase should have resulted in an equilibrium atmospheric warming of $0.33 \pm 0.17^\circ\text{C}$ based on a logarithmic interpolation from an assumed doubling response of $3.0 \pm 1.5^\circ\text{C}$ derived theoretically¹⁹. The predicted response

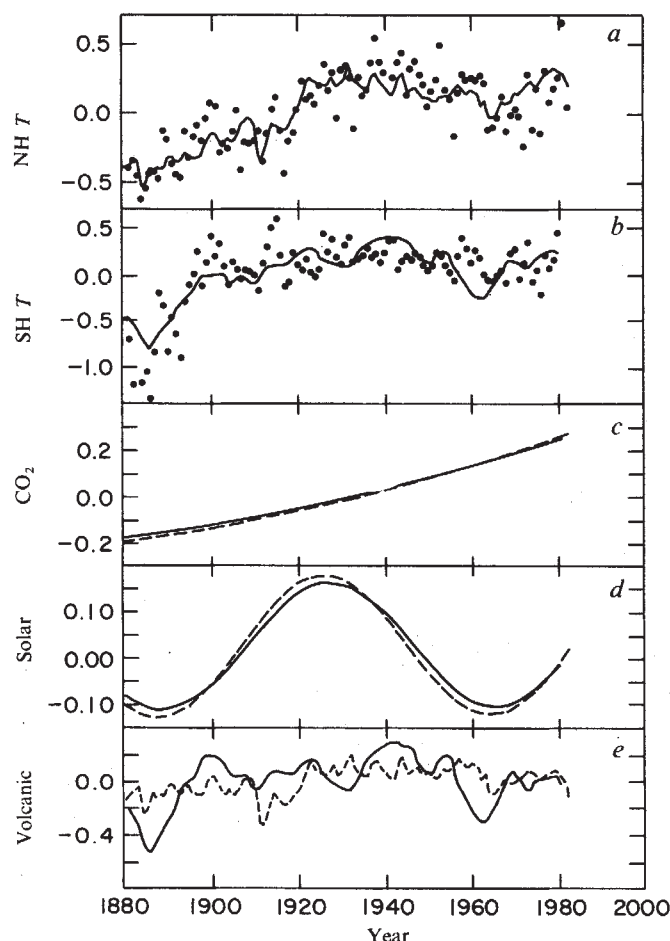


Fig. 1 Model simulation results for case with CO_2 (initial) at 260 p.p.m., volcanic perturbations based on ice core records of acidity (north) or sulphate (south) and 'solar' forcing through a 76-yr harmonic term (model 3 of Table 1). *a, b*, The model output (solid line) and observed temperatures (arbitrary zero point) for air over land in the Northern (NH) and Southern (SH) Hemispheres. *c, d, e*, The northern (dashed line) and southern (solid line) hemispheric responses to the individual forcings of CO_2 , solar and volcanic activity, respectively.

over a short interval may be reduced by the thermal inertia of the system. The expected increase of temperature over the last century of a few tenths of a $^{\circ}\text{C}$ is large enough compared with the several tenths of a $^{\circ}\text{C}$ observed fluctuations (see Fig. 1) to raise hope of its empirical detection. Unfortunately (from the point of view of providing simple confirmation), average temperatures for the Northern Hemisphere land stations were decreasing over the years 1940–70. Estimates of the mid-nineteenth century base level of CO_2 range from 260 to 300 p.p.m. with lower values gaining favour in recent research^{20,21}. We shall adopt a range of concentrations for AD 1850 starting at 260 and 300 p.p.m. (see Fig. 2*a*) to test the sensitivity of empirical derivations of CO_2 warming to the large uncertainty in the history of this forcing.

Stratospheric injections of sulphur compounds from large volcanic events lead to the production of sulphuric acid particulates, which scatter incident sunlight and induce cooling in lower atmospheric layers. Although there has been recently a general convergence of theoretical and empirical evidence for the existence of a volcanic influence on climate^{14–17}, details are speculative. For example, alternative reconstructions^{22,23} of Northern Hemisphere volcanic dust indices show large discrepancies over the 1940–60 period of crucial significance for this study and may include events added subjectively and based on prior knowledge of anticipated climatic effects. An objective measure of stratospheric sulphate loading exists in the records

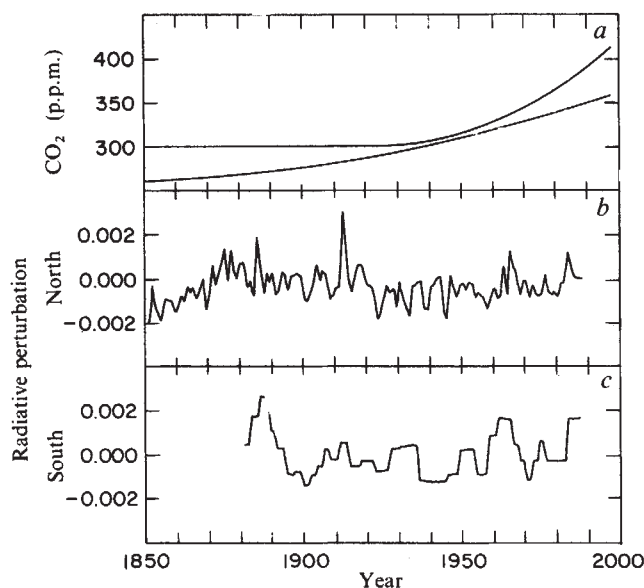


Fig. 2 *a*, Two possible records of increase (p.p.m.v.) for CO_2 . The curve starting at 260 p.p.m. passes through the observational record from 1958 to 1980, while the curve starting at 300 p.p.m. shows a more rapid increase which roughly mimics the combined increase of other greenhouse gases in addition to CO_2 . *b*, Fractional radiative perturbations derived from an acidity trace from a Greenland ice core²⁴ (representing fallout of stratospheric sulphates) through 1972 with an extension through 1981 based on direct stratospheric sampling for sulphates³¹. The radiative perturbation for the Northern Hemisphere (*c*) is based on sulphate content of an Antarctic ice core²⁵. Extensions beyond 1981 in *b* and *c* assume that the El Chichon event matches that of Agung in 1963—lack of observational temperature records beyond 1982 and 1980 for the Northern and Southern Hemispheres respectively, makes the details of these extensions unimportant for the model fits.

of acidity or sulphate content from polar ice cores^{24,25} in respective hemispheres. Despite the many caveats¹ which apply when using acidity or sulphate traces as indicators of hemispheric-averaged volcanic dust veils, they are probably the best such records available, although the relative error content may be large and is not adequately understood. Figure 2 *b, c* shows plots of the volcanic forcings used in this study.

Justification for inclusion of a solar term follows the finding^{26,27} of solar diameter variations with a period of some 76 yr and the theoretical expectation²⁸ that luminosity changes could accompany diameter variations, but with largely unconstrained phase and amplitude relations. Because no sufficiently precise direct measures of the solar luminosity as yet exist over long enough time scales to constrain our expectations, this suggested climate forcing is clearly hypothetical. In selected experiments we shall allow a sinusoidal variation of the solar constant at a period of 76 yr. A condition for this hypothesis will be whether or not the phase and amplitude of this harmonic component of climatic change agree in separate tests for the Northern and Southern Hemispheres.

The energy balance climate model discussed above is integrated forward in time using 1-day time steps starting from assumed equilibrium initial conditions in 1850. A time-dependent perturbation to the incoming radiation is applied for the volcanic (yearly resolution) and solar terms and to the outgoing IR loss term to account for changing CO_2 . Amplitude and phase shift (where applicable) factors for the perturbations are determined in a least-squares sense by fitting the appropriate model output temperature time series to its corresponding observed record beginning in 1881 (that is, hemispheric air temperature over land).

Table 1 presents for climate simulations of the past century fitting the Northern and Southern Hemispheres both separately and simultaneously using forcing functions that are sometimes

Table 1 Climate simulations of the past century for the Northern and Southern Hemispheres

Model no.	Model forcing factors		Domain of fit		Volcanic amplitude*		Solar†	Phase	2×CO ₂ ‡	r§	
	1850 CO ₂	76-yr solar	N	S	N	S	Amplitude	(yr)	Equilibrated amplitude	N	S
1	260	Yes	N		2.07 (37)		0.30 (22)	-16.6	1.36 (31)	0.825	
2	260	Yes		S		2.83 (40)	0.40 (18)	-4.8	1.89 (20)		0.795
3	260	Yes	N	S	2.25 (39)	3.02 (41)	0.30 (12, 17)	-11.4	1.53 (32, 19)	0.808	0.778
4	300	Yes	N	S	2.16 (38)	2.96 (41)	0.30 (11, 17)	-10.8	1.60 (32, 19)	0.802	0.781
5	260	No	N	S	2.92 (44)	3.90 (43)			1.22 (30, 17)	0.737	0.736
6	260	No	N						1.77 (34)	0.582	

* Multiplicative factor applied to perturbations shown in Fig. 2. Numbers in parentheses for this and latter columns show per cent variance explained by this component.

† Amplitude is peak-to-peak % variation of 76-yr harmonic. Phase is with respect to peak in 1911.

‡ Empirically derived response in °C for an equilibrated doubling of CO₂ assuming a logarithmic CO₂-temperature relationship.

§ Linear correlation coefficients between observed temperatures and model simulations.

hemispheric and sometimes global. Modelling a real global forcing term (CO₂ and solar) should yield similar characterizing parameters independent of the hemisphere which is simulated, if the term—or one proportional to it—is, in fact, operative in nature (and assuming the response characteristics of both hemispheres are qualitatively similar). Volcanic forcing is less global in extent, and may well have substantially different effects in the two hemispheres, results which can be partially tested by using independent, but simultaneous volcanic forcing records for the Northern and Southern Hemispheres.

Models 1–3 of Table 1 show the results of climate simulations with initial CO₂ at 260 p.p.m. separate volcanic forcing records from polar region ice cores and a harmonic component at 76 yr; results for entry 3 are shown in Fig. 1. An interesting result is the consistency of the 76-yr harmonic independently derived for the two hemispheres as shown by models 1–3 of Table 1. Whether or not the 76-yr harmonic is solar forced, these results suggest that an important common component of climatic change not associated with CO₂ or volcanic forcings exists in both hemispheres. The most obvious identification appears to be solar, especially when the plausibility arguments^{26–28} that the Sun is variable at this time scale are taken into account. Model 5 (compare with 3) shows the effect of not allowing for a 76-yr harmonic component.

Sensitivity to different CO₂ concentration scenarios beginning at 260 and 300 p.p.m. is shown by models 3 and 4 of Table 1. The greenhouse-induced temperature increase from 1880 to 1982 is 52% larger for the low versus the high baseline value, while the predicted doubling response is smaller by only 13%. In general, results for the Northern and Southern Hemisphere simulations yield surprisingly consistent results. The correlation coefficient for the Northern Hemisphere is invariably higher than for the Southern, which may reflect the better databases available for the Northern Hemisphere, but the difference is not meaningful given all the uncertainties. Fitting only CO₂ (model 6 of Table 1) results in much less explained total variance, than with the combined fits.

The results of this study are generally consistent with, but lower than, most theoretical expectations for CO₂-induced warming. In particular, the simulations of Table 1 show a mean doubling response of 1.6 ± 0.3 °C, in rough agreement with the lower range of theoretical predictions summarized in a recent US National Academy of Sciences report¹⁹. It is thus possible to claim cautiously, as others have previously, that a surface warming due to the greenhouse effect appears quite consistent with the observational climate record. It is, however, not yet possible to be quantitative and it is not valid to attach any statistical significance to the above claim. This is because there are still too many tunable parameters in our models and too few quantitative observations of external climatic forcings over the past century to make meaningful statistical significance tests. Moreover, if our model had different parameter values or functional forms for parameterization of internal feedback processes

or thermal capacities²⁹, it is conceivable that our fits in Table 1 could be quite different. On the other hand, the higher (about 4 °C) CO₂ doubling equilibrium response of recent general circulation model calculations²⁹ could be a result of too strong a set of positive feedback mechanisms (such as cloudiness parameterization) in the GCMs. Or, it is also conceivable that the equivalent surface temperature doubling response we find semi-empirically for the about 20% increase of CO₂ from 1890 to 1980 actually is about 1.6 °C, while at the same time an actual doubling of CO₂ would cause an equilibrium surface response of about 4 °C because of nonlinear feedback processes. It is impossible to resolve these uncertainties at present. However, based on the seasonal cycle tests⁵, we claim that the basic structure of our model is at least reasonable to a first order. Therefore, barring some dramatic new counter evidence, the advent of a significant greenhouse warming in future decades should not come as a surprise^{1,2,19,30}. The magnitude, though, is still unclear to a rough factor of 2.

An interesting counterpoint²³ to increasing greenhouse gases is the possibility³¹ that ambient stratospheric sulphate is increasing at 6–8% yr⁻¹, implying doubling times of 12–9 yr. The current background of stratospheric sulphates produces climatically-negligible extinction, but increases will produce linear perturbations (unlike CO₂ for which the global temperature response is logarithmic due to saturation of certain absorption bands). Effects to date are included in the modelling through the *in situ* results³¹ used to extend the 'volcanic' forcing record past 1972 in the Northern Hemisphere. At a doubling time of 10 yr for anthropogenic sulphate releases, the background stratospheric sulphate level would increase to that of large volcanic events (Agung, El Chichon) in 3–4 decades, at which point the sulphate-induced cooling would offset projected greenhouse warming. Clearly, continued monitoring of stratospheric sulphate concentrations is important.

There are at least two ways of making further progress in detecting the CO₂-induced global-scale climate warming. (1) Wait a few decades until the warming signal is unambiguous. This, of course, presupposes that a warming is occurring at predicted rates and that other simultaneous effects are unimportant. But even after some decades it will be possible that other natural causes could have induced a warming of several tenths of a °C, as had occurred earlier this century³². (2) Reduce the uncertainty associated with other causes of climatic change (such as volcanoes, possibly solar variations, and other trace gases) which are simultaneously operative. This would allow the known effects to be directly accounted for in the observed record, reducing the number of free parameters to be fit and improving the chances of obtaining a statistically significant result for residual effects like CO₂. We believe that this approach is preferable as it allows for both prediction and physical understanding—the latter being important for the credibility of any anticipated response by society to CO₂ increases³³. Through studies which attempt to include quantitatively a plausible set of

climatic forcings we can hope to at least elucidate the fruitful areas for more specialized research, if not bound a plausible range of CO₂-induced climatic responses. In this respect, accurate monitoring of plausible climatic forcings such as total solar irradiance or stratospheric aerosol loadings are essential.

Although we make no claim for statistical significance in our calculations, we have emphasized the need for additional independent verification of 76-yr solar and interhemispheric volcanic forcings. We have also suggested that comparisons between model and observational data for the purpose of detecting a possible CO₂ signal empirically are more meaningful when

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- Gilliland, R. L. *Climatic Change* 4, 111–131 (1982).
- Schneider, S. H. & Thompson, S. L. *J. geophys. Res.* 86, 3135–3147 (1981).
- Hansen, J. *et al. Science* 213, 957–966 (1981).
- Harvey, L. D. D. & Schneider, S. H. *J. geophys. Res.* (in the press).
- Harvey, L. D. D. & Schneider, S. H. *J. geophys. Res.* (in the press).
- Oort, A. H. & Vonder Haar, T. H. *J. phys. Oceanogr.* 6, 781–800 (1976).
- Schutz, C. & Gates, W. L. *Global Climate Data for Surface, 800 mb, 400 mb: October* (The Rand Corporation, R-915-ARPA, 1974).
- Esbensen, S. & Kushnir, Y. *Climate Research Institute Rep. No. 29* (Oregon State University, 1981).
- Schneider, S. H. & Mass, C. *Science* 190, 741–746 (1975).
- Jones P. D., Wigley, T. M. L. & Kelly, P. M. *Mon. Weath. Rev.* 110, 59–70 (1982).
- Neftel, A., Oeschger, H., Schwander, J., Stauffer, B. & Zumbunn, R. *Nature* 295, 220–223 (1982).
- Thompson, S. L. & Schneider, S. H. *Nature* 290, 9–10 (1981).
- Eddy, J. A., Gilliland, R. L. & Hoyt, D. V. *Nature* 300, 689–693 (1982).
- Mass, C. & Schneider, S. H. *J. Atmos. Sci.* 34, 1995–2004 (1977).
- Taylor, B. L., Gal-Chen, T. & Schneider, S. H. *Q. J. R. met Soc.* 106, 175–199 (1980).
- Pollack, J. B. *et al. J. geophys. Res.* 81, 1071–1081 (1976).
- Hansen, J. E., Wang, W. C. & Lacis, A. A. *Science* 199, 1065–1068 (1978).
- Keeling, C. D., Bacastow, R. B. & Whorf, T. P. in *Carbon Dioxide Review: 1982* (ed. Clark, W. C.) 377–385 (Oxford University Press, New York, 1982).
- National Research Council *Carbon Dioxide and Climate: A Second Assessment* (National Academy Press, Washington DC, 1982).
- Wigley, T. M. L. *Climat. Change* 5, 315–320 (1983).
- Schneider, S. H. *Climat. Change* 5, 311–313 (1983).
- Lamb, H. H. *Phil. Trans. R. Soc.* 266, 425–533 (1970).
- Mitchell, J. M. Jr in *The Changing Global Environment* (ed. Singer, S. F.) 149–173 (Reidel, Massachusetts, 1975).
- Hammer, C. U. *Nature* 270, 482–486 (1977).
- Delmas, R. & Bouteon, C. *J. geophys. Res.* 85, 5645–5649 (1980).
- Gilliland, R. L. *Astrophys. J.* 248, 1144–1155 (1981).
- Parkinson, J. H., Morrison, L. V. & Stephenson, F. R. *Nature* 288, 548–551 (1980).
- Gilliland, R. L. *Astrophys. J.* 253, 399–405 (1982).
- Hansen, J. *et al. in Climate Processes and Climate Sensitivity, Maurice Ewing Series Vol. 5* (eds Hansen, J. E. & Takahashi, T.) (American Geophysical Union, Washington DC, 1984).
- Thompson, S. L. & Schneider, S. H. *Nature* 295, 645–646 (1982).
- Sedlacek, W. A., Mroz, E. J., Lazrus, A. L. & Gandrud, B. W. *J. geophys. Res.* 88, 3741–3776 (1983).
- Wigley, T. M. L. & Jones, P. D. *Nature* 292, 205–208 (1980).
- Schneider, S. H. & Londer, R. *The Coevolution of Climate and Life* (Sierra Club, San Francisco 1984).

Graphite crystals in hydrothermal vents

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Most studies of the hydrothermal input at active ocean ridges have concentrated on elements present in solution rather than particulate matter. During the exploratory cruises Clipperton^{1,2} and Geocytherm³, we moored and recovered sediment traps close to hydrothermal vents associated with the 13° N East Pacific Rise (EPR) with the aim of studying the particulate flux. The first trap was moored about 300 m east of the axis of the central valley, the second was located directly in the central valley at about 200 m from active vents. Both traps were 50 m above the sea floor and well under the depth of expansion of the hydrothermal plume¹. We report here that, in addition to the expected sulphides, we recovered crystals of graphite with antimonide overgrowths. This graphite is considered to be of hydrothermal origin; it can readily be distinguished from continentally-derived carbonaceous particles. The flux of hydrothermal graphite is estimated to be about 1.7 nmol cm⁻² yr⁻¹.

The sediment traps were released and recovered using techniques developed by Honjo⁴ so as to avoid pollution of the samples. Several size fractions and aliquots were obtained, but the observations reported here were made on the <1-mm size fraction from both traps. The particles were transferred and fixed onto the membrane filters. The methods that we used to scan the membrane filters and to select and analyse interesting objects have been outlined elsewhere^{5,6}. The total sediment collected was about 300 mg for CL/ST1 during a 10-day deployment, and about 7 g for CL/ST2 during a 222-day deployment.

The particles contain most of the mineral species, such as pyrrhotite, chalcopyrite, iron sulphides, zinc sulphides, calcium sulphates, barite and silica that previous investigators have

distinctions are made between air and surface temperatures, and between land and oceanic domains. And when such distinctions are made, as done simply here, we still find that most of the variance of long-term surface air temperatures over land can be accounted for by volcanic, CO₂ and solar forcing for the past century.

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- Keeling, C. D., Bacastow, R. B. & Whorf, T. P. in *Carbon Dioxide Review: 1982* (ed. Clark, W. C.) 377–385 (Oxford University Press, New York, 1982).
- National Research Council *Carbon Dioxide and Climate: A Second Assessment* (National Academy Press, Washington DC, 1982).
- Wigley, T. M. L. *Climat. Change* 5, 315–320 (1983).
- Schneider, S. H. *Climat. Change* 5, 311–313 (1983).
- Lamb, H. H. *Phil. Trans. R. Soc.* 266, 425–533 (1970).
- Mitchell, J. M. Jr in *The Changing Global Environment* (ed. Singer, S. F.) 149–173 (Reidel, Massachusetts, 1975).
- Hammer, C. U. *Nature* 270, 482–486 (1977).
- Delmas, R. & Bouteon, C. *J. geophys. Res.* 85, 5645–5649 (1980).
- Gilliland, R. L. *Astrophys. J.* 248, 1144–1155 (1981).
- Parkinson, J. H., Morrison, L. V. & Stephenson, F. R. *Nature* 288, 548–551 (1980).
- Gilliland, R. L. *Astrophys. J.* 253, 399–405 (1982).
- Hansen, J. *et al. in Climate Processes and Climate Sensitivity, Maurice Ewing Series Vol. 5* (eds Hansen, J. E. & Takahashi, T.) (American Geophysical Union, Washington DC, 1984).
- Thompson, S. L. & Schneider, S. H. *Nature* 295, 645–646 (1982).
- Sedlacek, W. A., Mroz, E. J., Lazrus, A. L. & Gandrud, B. W. *J. geophys. Res.* 88, 3741–3776 (1983).
- Wigley, T. M. L. & Jones, P. D. *Nature* 292, 205–208 (1980).
- Schneider, S. H. & Londer, R. *The Coevolution of Climate and Life* (Sierra Club, San Francisco 1984).

identified in hydrothermal chimneys and basal mounds^{3,7,8}. Several antimonides were also recognized, and their presence is important for attributing an origin to the graphite particles. The following compositions were obtained: Sb-S; Pb-Sb-S; Fe-Sb-S; Pb-Fe-Sb-S; Pb-Fe-Sb-As-S (Cu and Zn are commonly minor components).

During our study of what looked like antimonides, we came across several grey, opaque particles which did not display the characteristic lines of Sb and S when scanned. They were eventually identified as graphite particles (Fig. 1) and commonly contained overgrown stibine (Sb₂S₃) and various Fe-Cu-Pb-sulphoantimonides (Fig. 2). All these antimonides are also found as loose fragments and crystals in the trap's sediments, but are never deposited on biogenic debris. Thus, this paragenetic association of graphite and antimonides can be considered characteristic of the deep-seated origin of the graphite. Although the presence of continental carbonaceous particles in oceanic sedimentation⁹, is a complication inherent to the collection device, it does not create a problem when distinguishing between the two types of carbonaceous particles (deep-seated and continental), provided microscopical (such as hue and morphology) and not chemical criteria are used¹⁰.

Taking into account the results from similar sediment traps^{4,11–14}, it seems that this occurrence of graphite is related to the vicinity of hydrothermal vents.

The number of graphite particles gave flux estimates which are in the same range for both sediment traps: 3.5–7 particles cm⁻² yr⁻¹ for CL/ST1 and 3.2–6.4 particles cm⁻² yr⁻¹ for CL/ST2. With an average particle size of 15 µm, the corresponding average flux of carbon as graphite should be 1.7 nmol cm⁻² yr⁻¹, or 20.4 ng cm⁻² yr⁻¹. As graphite carbon is probably unreactive in seawater, this also represents the flux sedimenting in the vicinity of the hydrothermal vents at 13° N EPR. But an indiscriminate count of all carbonaceous particles, a chemical measurement of the total elementary carbon in sediments¹⁰ would be wrong, since carbons from different origins are added together.

In the same deployments, we have measured the fluxes of several elements introduced by hydrothermal activity (J.B. *et al.*, in preparation). The flux of carbon is smaller than that of Fe, 1,100; Mn, 13; or Cu, 6 nmol cm⁻² yr⁻¹ but is in the same range as Ba, 3, and much larger than Sb, 0.04 nmol cm⁻² yr⁻¹.

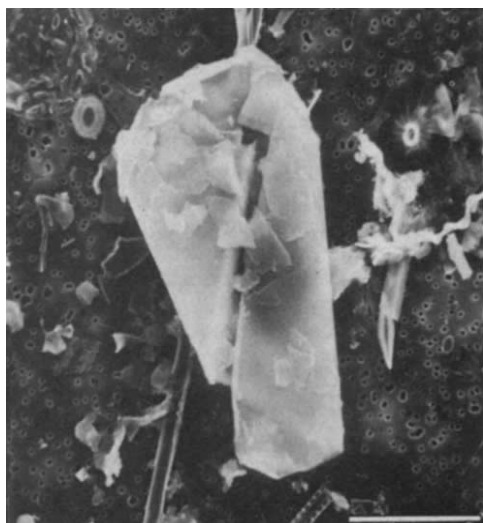


Fig. 1 Particle 3 (CL/ST2). Large graphite crystal of angular outline with small surficial platelets, also of graphite (scanning electron microscopy (SEM), secondary mode). Scale bar, 10 μm . The features of the graphite particles and methods used are summarized below. Morphology under light and SEM reveal an irregular outline with some straight edges. Regular crystals are not present. Particles are generally flattened. Texture under light and electron microscopes show stackings of irregular lamellae, often kinked. Terminal platelets are overgrown with smaller platelets. The particles are between 5 and 100 μm with most between 5 and 20 μm , and an average of 15 μm . Particles <2 μm could also be present. Optical properties under the light microscope and oil immersion: (1) transmitted light is fully opaque; (2) reflected light, unpolarized, is moderately reflecting in blueish grey; (3) reflected light, fully polarized, is anisotropism visible only along the kinks. As graphite is one of the most anisotropic substances, we checked the optical properties of unpolished particles, observed normally to the basal cleavage, on graphite from Buckingham, Ontario. The latter matched the properties of our oceanic particles. These can also be easily distinguished from continental, fire-generated carbonaceous particles by hue, anisotropy and morphology: the continental carbons display a characteristic bronze hue, distinct reflection pleochroism and birefractance and various characteristic morphologies: moss, hollow spheres, splinters, punctuated ribbons⁹, but no stacked platelets. The chemical composition determined by electron microprobe showed (1) energy dispersive spectrometer: humped spectrum, with hump at 1–2 KeV; minor elements: Si, Cl, Ca, Fe. (2) Wavelength dispersive spectrometer with a lead stearate crystal reveals a strong carbon line at 52.71° (2 θ). The intensity of the carbon line is consistent with graphite, when using CaCO₃ (coccoliths) and Buckingham graphite as standards. The pattern obtained with 30 analysed particles using X-ray diffraction with the Chesley micro-camera, extracted by micromanipulation, consists of 1 line and several spots corresponding to the 2H polymorph of graphite; but the data cannot rule out the possible presence of the 4H or/and 3R polymorphs, as reflections other than the strong (002) are found as spots which were unsuitable for intensity measurements.

The presence of graphite in the hydrothermal input to the ocean can be related to other observations of ridge systems. Carbon graphite has been observed in submarine basalts^{15,16}. An estimate of the relative concentration of graphite can be obtained from carbon extracted in fusion experiments; at 13° N, the concentration obtained for carbon in basalt is 246 ± 20 p.p.m. which is high for ridge systems¹⁶. Graphite can be produced from three main processes: (1) disproportionation of CO: $2\text{CO} \rightarrow \text{C} + \text{CO}_2$, which is catalysed by sulphides¹⁵; (2) reduction of CO: $\text{CO} + \text{H}_2 \rightarrow \text{C} + \text{H}_2\text{O}$, which is catalysed by Fe (ref. 15); (3) breakdown of CO₂: $\text{CO}_2 \rightarrow \text{C} + \text{O}_2$, which implies the intermediary formation of CO (ref. 17), and must be followed by the reduction of O₂ (by H₂, H₂S, Fe, Fe²⁺, ...) for the reaction to occur. In any case reducing agents are necessary. Observations of the serpentinization of olivine^{18,19} show that reducers such

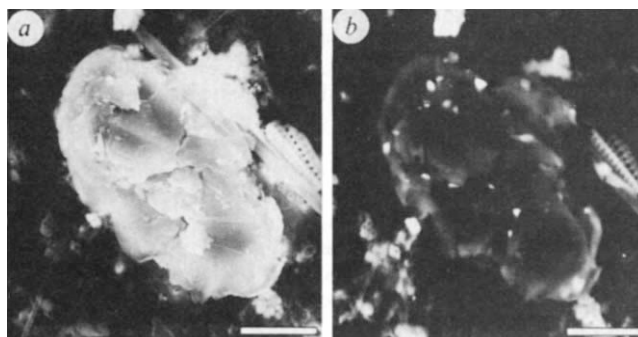
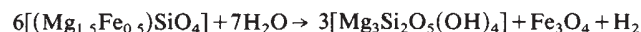
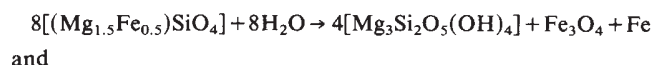


Fig. 2 Particle 36 (CL/ST2). *a*, Graphite particle of rounded outline. (SEM, secondary mode). *b*, As *a* but in reflective mode. The highly reflecting particles are sulpho-antimonides concentrated on the graphite particle. Scale bars, 10 μm .

as Fe and H₂ are involved. The reactions are respectively:



In this case, graphite is associated with metallic iron and serpentine, as found in "alpine peridotite type"¹⁸.

This possibility is sustained by the presence of metallic iron associated with (deposited on) silica, as found in the sediment trap (Fig. 3). Only two such particles were observed in the scanned filters from CL/ST1 (data not shown). However, this is significant as one would not expect to find metallic iron in sediment traps owing to its high reactivity in seawater. Its presence must be transient and sustained only due to the production and input of the vent system. Silica may have acted as protective coating for iron, and the short recovery time of ST/CL1 may have been crucial for the good state of preservation of unaltered minerals of the vents (J.B. *et al.*, in preparation). Octahedral magnetite (Fe₃O₄) has also been observed in both sediment traps and as a minor phase of the chimney⁸.

That CO or CO₂ is the most probable source of carbon graphite is difficult to prove. In the temperature range 350–500 °C, CO₂ has a higher concentration than CO in the C–O–H–S system²⁰. At 13° N, the CO₂/CO volume ratio is 80 in the basalt vesicles¹⁶. This may mean that CO has reacted, yielding C and additional CO₂, or that CO₂ is the most probable source.

Thus, we propose that the graphite crystals recovered in the vicinity of hydrothermal vents are formed during serpentinization by reaction of CO and/or CO₂. Some particles can be transported by hydrothermal waters and released in seawater. Because graphite is quite unreactive in seawater, it will be found unaltered in the vicinity of the vents. Although very low, the flux of graphite should be measurable in sediments as it is also stable in that environment. Hence, an assessment of the sedimentary flux of crystalline graphite can be used to estimate the hydrothermal input to the oceans and its variations. One must take into account the contribution of continental derived carbon particles, which have different properties⁹ from hydrothermal carbon graphite and can thus be distinguished.

The presence of crystalline graphite also suggests that deep-seated hydrothermal solid products may be transported in the hydrothermal plumbry, as can also be predicted from observations of hydrothermal chimneys²¹. Although witnesses of the black smokers have often reported that there are no particles in the fluid at its outlet, it would be difficult to observe micrometre-range particles with velocity in the range 0.5–2 m s⁻¹ (ref. 3). Thus the identity of what is measured (dissolved, precipitates) in hydrothermal fluids should be examined to find out which elements are involved in deep-seated reactions (silica, metals).

Graphite particles are considered to be efficient scavengers of organometallic compounds. This makes them suitable for the

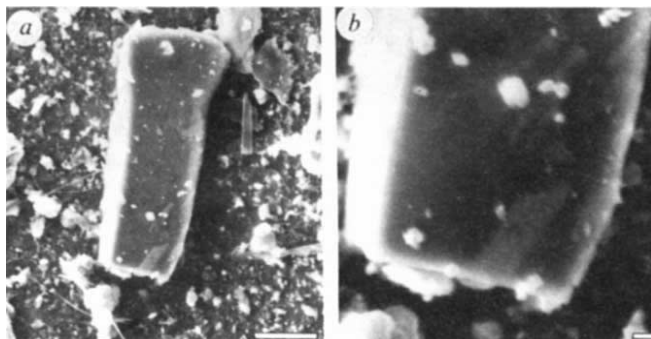


Fig. 3 Particle 34 (CL/ST1). Angular silica particle overgrown with metallic iron. *a*, Silica particle (SEM, secondary mode). *b*, Enlargement of the lower part of *a*. The subparallel overgrowths of metallic iron appear in light grey at the lower and upper right corners. Scale bars: *a*, 10 μ m; *b*, 1.0 μ m.

fixation of bacterial life in the vicinity of hydrothermal vents, and for its continuation in metalliferous sediments produced through vent activity.

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- Hékinian, R. *et al.* *Science* **219**, 1321–1324 (1983).
- Boulègue, J., Lafitte, M. & Maury, R. *C.R. hebdom. Séanc. Acad. Sci., Paris* **296**, 1729–1732.
- Hékinian, R. *et al.* *Mar. Geophys. Res.* **6**, 1–14.
- Honjo, S. *J. mar. Res.* **38**, 53–97 (1980).
- Jedwab, J. *Geochim. cosmochim. Acta* **43**, 101–110 (1979).
- Jedwab, J. *Earth Planet. Sci. Lett.* **49**, 551–564 (1980).
- Haymon, R., Kastner, M. *Earth Planet. Sci. Lett.* **53**, 363–381 (1981).
- Oudin, E. *Mar. Min.* **4**, 39–73 (1983).
- Smith, D. M., Griffin, J. J. & Goldberg, E. D. *Nature* **241**, 269–270 (1973).
- Smith, D. M., Griffin, J. J. & Goldberg, E. D. *Analyt. Chem.* **47**, 233–238 (1975).
- Dymond, J. *et al.* *Earth Planet. Sci. Lett.* **53**, 409–418 (1981).
- Brewer, P. G., Nosaki, Y., Spencer, D. W. & Fleer, A. P. *J. mar. Res.* **38**, 703–708 (1980).
- Honjo, S., Manganini, S. J. & Cole, J. J. *Deep Sea Res.* **29**, 609–625 (1982).
- Cobler, R. & Dymond, J. *Science* **209**, 801–803 (1980).
- Mathez, E. A. & Delaney, J. R. *Earth Planet. Sci. Lett.* **56**, 217–232 (1981).
- Pineau, F. & Javoy, M. *Earth Planet. Sci. Lett.* **62**, 269–270 (1983).
- French, B. M. *Rev. Geophys.* **4**, 223–253 (1966).
- Ramond, P. *Neues Jb. Miner. Abh.* **107**, 241–265 (1967).
- Pasteris, J. D. *Geology* **9**, 356–359 (1981).
- Gerlach, T. M. & Nordlie, B. E. *Am. J. Sci.* **275**, 353–376 (1975).
- Lafitte, M., Maury, R., Perseil, E. A. & Boulègue, J. *Earth Planet. Sci. Lett.* (in the press).

Anomalous focal mechanisms: tensile crack formation on an accreting plate boundary

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Conventional double-couple solutions do not satisfactorily account for many small magnitude earthquakes in the Hengill geothermal area in the Neovolcanic Zone of Iceland. The far-field radiation pattern is predominantly compressional but a few dilatational arrivals have been recorded. We prefer the interpretation that these events are due to tensile crack formation at a shallow (1–7 km) depth within a cooling intrusive body. Volume considerations indicate that initial fracturing of the rock is seismic but that subsequent widening proceeds aseismically. Other reports of anomalous radiation patterns from earthquakes on the Mid-Atlantic Ridge describe events of a similar type, that is, exhibiting predominantly compressional radiation patterns and a reduced dilatational field^{1–4}.

In 1981, an intensive 3-month seismic monitoring project was carried out in the Hengill geothermal area of Iceland (Fig. 1). The project was designed using foreknowledge of the spatial distribution of earthquake activity within the area and its ongoing nature⁵. The crustal structure is well constrained by refraction shooting^{6,7} and shots fired within the area during the monitoring project confirmed that the crustal model used is accurate to within 5%. In total, 30 seismometers were operated within 14 km of the centre of the network.

About 140 well-constrained focal mechanism solutions were obtainable for small magnitude earthquakes occurring within the network. Conventional double-couple solutions with orthogonal nodal planes cannot be fitted to half of the events. Compressional arrivals dominate the focal sphere and dilatational arrivals of normal amplitude project onto the focal sphere in a narrow zone striking about north-east. Two examples and a composite solution are shown in Fig. 2. The dilatational and compressional portions of the focal sphere may be separated by small circles with typical radii of about 80°. Hence the ratio of the principal seismic moments of these events is approximately: 30:–1:–1.

The predominant component of this mechanism is a linear vector dipole (LVD). The sum of the principal moments is significantly greater than zero, indicating a volume increase. In this respect, the source differs fundamentally from the CLVD source recognized by Julian⁸.

We propose that this radiation pattern is generated by the formation of a tensile crack in the presence of a pore fluid, and that this type of fissure formation would be expected to occur in the tensile stress regime of an accreting plate boundary.

The Hengill area forms part of the continuation of the Mid Atlantic Ridge (MAR) onto the Icelandic landmass and has been volcanically active for ~700,000 yr (ref. 9). Originally the volcanic centre lay to the north of the village of Hveragerdi but later moved west and now underlies the central volcano Hengill (Fig. 1). The area is occupied by an extensive and complex geothermal field, which is hottest in the immediate vicinity of

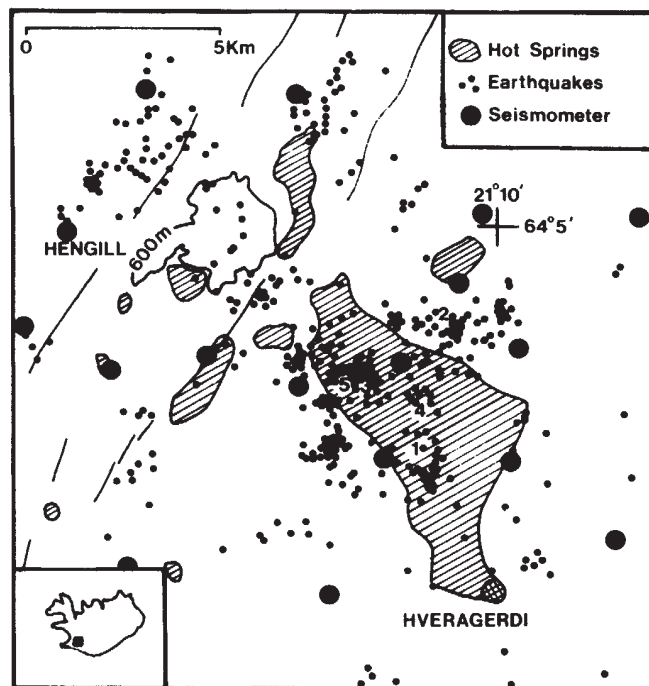


Fig. 1 Hengill central volcano and vicinity, the inset shows its location in Iceland. The map reveals seismic activity during the 3-month recording period, areas of hot springs and outline of fissure swarm transecting the area through Hengill. Of the 30 stations operated, 17 lie within the area shown. 1, 2, Locations of events shown in Fig. 2; 3, 4, 5, locations of events used for composite solution shown in Fig. 2.

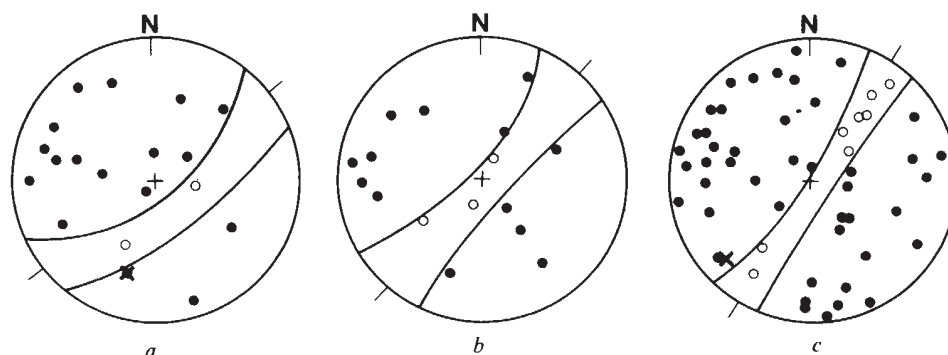


Fig. 2 P-wave first motions from two non-double couple events and one composite solution. Upper hemisphere plots in stereographic projection. ●, Compressions; ○, dilatations; ×, nodal arrivals; curved lines, small circles of radius θ defining nodal lines of tensile crack solution. *a*, Event 1: depth 4.87 km; $M_L = 0.1$; strike N 53° E; dip = 60°, $\theta = 78^\circ$. *b*, Event 2: depth = 4.99 km; $M_L = 0.0$; strike = N 42° E; dip = 80°; $\theta = 76^\circ$. *c*, Composite solution for three events; strike normalized; dip = 80°; $\theta = 80^\circ$.

Hengill. However, most of the hot springs, and hence the greatest heat loss, occur in the vicinity of the original volcanic centre. A fissure swarm striking at N 25° E transects the area, passing through Hengill. The eruption fissures, and vertical, open fissures contained within it, testify to the tensional nature of the stress regime of the area. The direction of least compressive stress is orientated N 65° W, normal to the fissure swarm¹⁰.

Earthquake activity is continuous. The activity rate is 1 magnitude (M_L) 0 event per day. The hypocentres of most of the events cluster in a volume beneath the old volcanic site. Hypocentral depths are mostly within the range 1–7 km (Fig. 3). The pore fluid is thus hydrous at shallow depths but may be magmatic deeper down. In such a regime, the most likely cause of continuous seismicity is contraction cracks in the cooling roots of the extinct volcano.

As a result of continuous heat loss from the surface, the rock cools and contracts. This produces a local tensional stress field that is superimposed on the regional. The total stress field is additionally modified by geothermal processes (for example, boiling in the rock), that influence the pore pressure on a very small scale. Where these processes result in an increase in pore pressure they may trigger fracture formation.

Failure in this predominantly tensile total stress field results in the formation of roughly vertical tensile cracks orientated about NE–SW.

The formation of a planar tensile crack in an isotropic medium would yield an all-compressive far-field radiation pattern of variable amplitude with respect to the orientation of the crack. The largest amplitude is normal to the crack, and the amplitude minimum traces a great circle on the focal sphere where the crack plane projects onto it. The point force equivalent of such a source may be represented by three mutually orthogonal vector linear dipoles with moments in the ratio: $(\lambda + 2\mu) : \lambda : \lambda$, where λ and μ are the Lamé elastic moduli¹¹. The seismic moment tensor of the Hengill events can be modelled by combining a tensile crack with a spherically symmetrical implosion:

$$[(\lambda + 2\mu) : \lambda : \lambda] + [-(\lambda + \delta) : -(\lambda + \delta) : -(\lambda + \delta)] \\ = [(2\mu - \delta) : -\delta : -\delta]$$

Fluid flow is a slow process compared with the speed at which a fracture forms so at the moment of fissuring, the volume occupied by the fluid in the immediate vicinity of a fracture increases and causes a local pore pressure drop¹². This sudden pore pressure drop is omnidirectional and generates the dilatational portion of the radiation pattern.

Decreasing pore pressure limits crack propagation and hence probably limits the size of events. The formation of one fracture causes local changes in the rock medium and stress field, and may trigger a chain reaction. This could explain the 'geothermal swarms' of numerous, very small events observed in many geothermal areas in Iceland.

It is, therefore, a pore pressure drop caused by restricted fluid flow at the moment of fissuring that generates the dilatational part of the radiation field and limits crack growth.

The natural heat loss of the Hengill area is 350 MW (ref. 13). If attributed to a cooling intrusion, the rate of volume contrac-

tion may be calculated using the equation:

$$\Delta V = \frac{H\gamma}{C_p\rho}$$

where ΔV is the contraction rate, H is the cooling rate, γ is the coefficient of thermal expansion ($\approx 16.2 \times 10^{-6} \text{ K}^{-1}$), ρ is the density ($\approx 3 \times 10^3 \text{ kg m}^{-3}$) and C_p is the specific heat of basalt at constant pressure ($\approx 1.3 \times 10^3 \text{ J kg}^{-1} \text{ K}^{-1}$). Application of this equation to the Hengill area gives a contraction rate of: $\Delta V = 4.5 \times 10^4 \text{ m}^3 \text{ yr}^{-1}$. An estimate of the total volume of contraction cracks formed seismically may be made and compared with this value. The equations^{14,15}:

$$\log M_0 = 15.1 + 1.7 M_L$$

$$\log E = 11.8 + 1.5 M_L$$

give estimates of seismic moment and energy release from local magnitudes (M_L). The ratio of principal seismic moments observed for the Hengill events indicates that δ is small. Therefore, they are predominantly linear vector dipole sources and seismic moment may be approximately related to volume by the equation: $M_0 = 2\mu V$, where V is the volume of the crack. Using these equations, the contraction rate within the seismically active volume is estimated to be two orders of magnitude smaller than that calculated for the cooling intrusion. A similar result is obtained if energy is considered.

These calculations demonstrate that the seismicity may be attributed entirely to a cooling volume and that it is likely that much aseismic widening occurs after the initial fracturing.

There have been other reports of anomalous radiation patterns from the MAR and its continuation onto the Icelandic land-mass^{1–4}. For these events, dilatational arrivals occupy less than

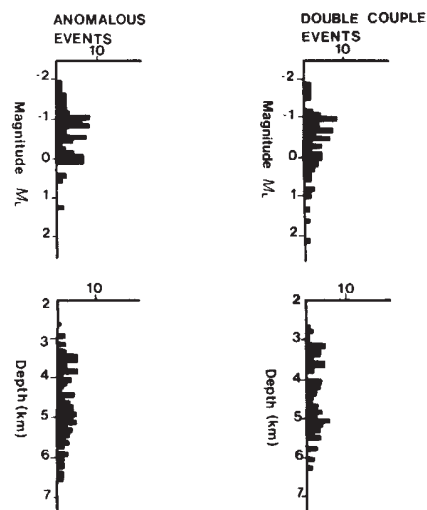


Fig. 3 Magnitudes and depths of events for which good focal mechanism solutions were possible.

half of the focal sphere. Interpreted in terms of source mechanism this also requires a net explosive component. In the case of large, teleseismically recorded events, where lower hemisphere polarity plots are made, explanations for the observed anomalous radiation patterns may readily be found by invoking interference, or path effects where the events are shallow and in areas of active volcanism, where severe crustal inhomogeneity is to be expected^{16,17}. However, in the Hengill case we are dealing with shallow earthquakes, upper hemisphere plots and a well constrained crustal structure. Many of the events exhibit extreme non-dipolarity and occur interspersed with double-couple sources. Here it is less easy to explain away the anomalous events as the result of a path effect.

We conclude that the Hengill source mechanism data indicate that at this point on the accreting plate boundary stress is being released by tensile crack formation. These data strongly suggest

that such sources can occur in nature in a tensile stress environment. Thus, more credence should be given to the theory that the anomalous radiation patterns reported for MAR earthquakes are due to source effects, particularly an enhanced explosive element leading to volume increase at source, as a result of the crustal accretion process.

Shear source mechanism solutions are commonly fitted to data that are not sufficient to constrain such a solution. The present results suggest that the custom of routinely fitting double-couple solutions to inadequate data should be viewed more critically.

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1. Sykes, L. R. *J. geophys. Res.* **72**, 2131–2153 (1967).
2. Sykes, L. R. *Bull. Seismol. Soc. Am.* **60**, 1749–1752 (1970).
3. Klein, F. W., Einarsson, P. & Wyss, M. *J. geophys. Res.* **82**, 865–888 (1977).
4. Einarsson, P. *Tectonophysics* **55**, 127–153 (1979).
5. Foulger, G. & Einarsson, P. *J. Geophys.* **47**, 171–175 (1980).
6. Palmason, G. *Crustal Structure of Iceland from Explosion Seismology* (Societas Scientiarum Islandica Publ. 40, 1971).
7. RRISP Working Group *J. Geophys.* **47**, 228–238 (1980).
8. Julian, B. R. *Nature* **303**, 323–325 (1983).

9. Björnsson, A., Tomasson, J. & Saemundsson, K. *Hengillsvaedid, stada jardhitarannsóknar vorið 1974* Orkustofnun Rep. OSJHD 7415 (1974).
10. Haimson, B. C. & Voight, B. *Pageophysics* **115**, 153–190 (1977).
11. Aki, Keiti & Richards, P. G. *Quantitative Seismology* (Freeman, San Francisco, 1980).
12. Robson, G. R., Barr, K. G. & Luna, L. C. *Nature* **218**, 28–32 (1968).
13. Bodvarsson, G. *J. Engnr Ass. Iceland* **36**, 1–48 (1951).
14. Wyss, M. & Brune, J. N. *J. geophys. Res.* **73**, 4681–4694 (1968).
15. Spottiswoode, S. M. & McGarr, A. *Bull. seismol. Soc. Am.* **65**, 93–112 (1975).
16. Solomon, S. C. & Julian, B. R. *Geophys. J. R. astr. Soc.* **38**, 265–285 (1974).
17. Trehu, A. M., Nabelek, J. L. & Solomon, S. C. *J. geophys. Res.* **86**, 1701–1724 (1981).

A complex of copper (II)-montmorillonite with a modified cyclodextrin

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Various classes of compounds, including hydroquinone, urea, cyclodextrin and montmorillonite, can act as host compounds having several types of inclusion spaces, such as three-dimensional cages, parallel channels and layers¹. Despite the variety of host compounds, however, little attention has been paid to the possible complexation of two or more of different kind host components: all inclusion compounds so far reported² use a single component as the host structure. We have now prepared, for the first time, a host compound consisting of two components, one layered and one single channel-like, in which dimers of 1:1 complex of Cu(II) with mono-(6-β-aminoethylamino-6-deoxy)-β-cyclodextrin (CDen) are formed in a stable condition and are closely packed between the silicate layers of montmorillonite, with their opening faces parallel to the interlayer surface. This compound is unique because the cyclodextrin which it takes up as a guest component can also act as host for many substances. In view of the enzymatic function of cyclodextrins, this class of compounds seems promising for use as an immobilized artificial enzyme and in models of membrane enzymes.

Cyclodextrins are cyclic oligosaccharides of D-glucopyranose. The CDen used in this study is a derivative of β-cyclodextrin, in which the 1,2-diaminoethane group is attached to a primary alcohol group on the narrow side of the truncated cone-shaped cyclodextrin molecule. The cyclic dextrins and their derivatives, including CDen, are very useful as micro-encapsulating agents or enzyme models as they stabilize sensitive volatile or toxic substances by encapsulating them, or catalyse various organic reactions in a manner similar to that observed in enzymatic reactions^{2,3}. The clay mineral montmorillonite is made up of negatively charged layers with a typical composition of (Al, Mg)₂₋₃(Si, Al)₄O₁₀(OH)₂ and interlayer cations compensating the positive charge deficiency of the silicate layers⁴. Due to

the layered structure, the clay mineral is capable of intercalating a variety of organic compounds.

CDen was prepared by the method described in ref. 5. The montmorillonite with a chemical composition



(from Tsukinuno mine, Yamagata, Japan) was placed in a 1.0 M CuCl₂ solution at 25 °C for 10 days to yield the Cu(II) exchanged form.

The inclusion behaviour of Cu(II)-montmorillonite towards CDen was examined by soaking the Cu(II) exchanged mineral in an aqueous CDen solution and measuring the contents of CDen and Cu(II) in the solid phase as a function of the CDen added⁶. As a result, a complex of Cu(II)-montmorillonite with CDen was found to form as a single phase when the CDen

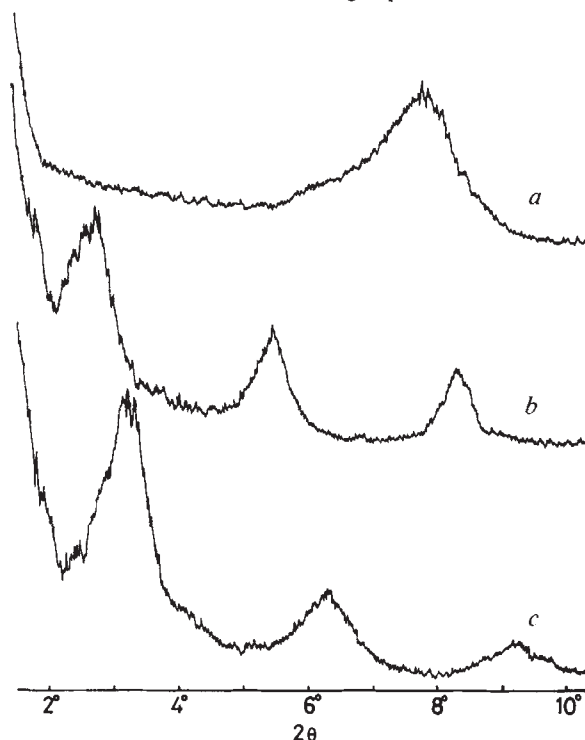


Fig. 1 X-ray powder diffraction patterns of a, Cu(II)-montmorillonite and b, wetted and c, dried forms of CDen-Cu(II)-montmorillonite (FeKα radiation).

added was more than 1.4 mmol g^{-1} ; the resulting solid was air-dried at 40°C . Figure 1 shows the X-ray diffraction patterns of the wetted and dried forms of the complex, together with that of Cu(II)-montmorillonite which has an interlayer spacing of $14.1 \text{ \AA}$. The interlayer spacings of the wetted and the dried forms of the Cu(II)-montmorillonite-CDen complex were 39.2 and $33.4 \text{ \AA}$, respectively. The amounts of CDen, Cu(II) ion and water in the dried solid were determined, respectively, as 0.58 , 0.55 and 6.7 mmol g^{-1} of clay by thermogravimetric or atomic absorption analysis. This indicates that the molar ratio of CDen to Cu(II) in the solid is $1:1$. The intercalated CDen cannot be removed by suspending the solid in water.

The dried CDen-Cu(II)-montmorillonite complex has expanded its layer by $23.9 \text{ \AA}$ more than that in the fully collapsed form of its inorganic component, $9.5 \text{ \AA}$ (ref. 7). This value is much less than twice the $15.4 \text{ \AA}$ for the van der Waals' diameter of the torus-shaped parent molecule but is very close to twice $11.8 \text{ \AA}$, which is the sum of the thickness of the torus and the extended chain length of the aminoethylamino group. This suggests that the CDen molecules would be intercalated as a bilayer with their opening faces parallel to the silicate layers of montmorillonite. Assuming that CDen molecules, each $15.4 \text{ \AA}$ in diameter, are hexagonally close-packed in the bilayer, the effective area per molecule is $2\sqrt{3} \times (15.4/2)^2$ or $205 \text{ \AA}^2$. As the unit cell weight of Cu(II)-montmorillonite is 752.6 and the surface area of one face of the unit cell is estimated as $46 \text{ \AA}^2$ (ref. 8), the CDen content can be evaluated as $2 \times (46/205)/752.6$ or $0.596 \text{ mmol g}^{-1}$ of clay, which is fairly close to the observed value.

The spectroscopic observation of an aqueous solution containing CDen and Cu(II) ions⁵ suggests that the intercalated CDen molecules might also form complexes with the Cu(II) ions that exist between the silicate layers. An electron spin resonance (ESR) measurement was made on a thin film of CDen-Cu(II)-montmorillonite that had been obtained by spontaneous resedimentation in water. The X-ray diffraction pattern taken perpendicular to the film plane did not show any peaks attributable to the (001) reflections, indicating that the silicate layers in the film are preferentially oriented parallel to the film plane. Figure 2 shows that the ESR spectrum of the film is hardly affected by the variation of angle between the film plane

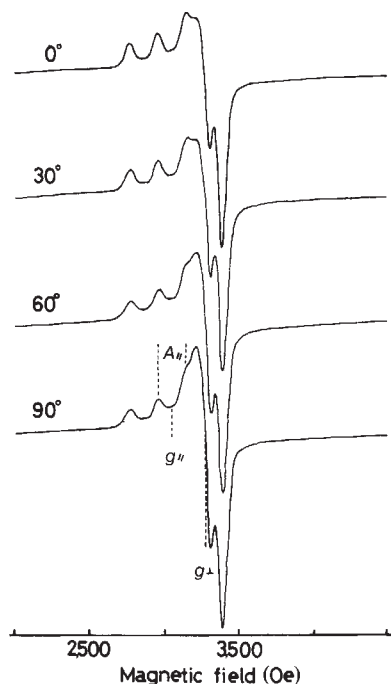


Fig. 2 ESR spectra of a thin oriented film of dried CDen-Cu(II)-montmorillonite as a function of the angle between the film plane and the magnetic field at 297 K . Microwave frequency, $9,424 \text{ MHz}$.

and magnetic field, and the combination of the spectrum with the powder pattern enables us to determine both perpendicular and parallel components of the g vector and absorbance A . The ESR parameters obtained are $g_{\perp} = 2.062$, $g_{\parallel} = 2.202$, $A_{\perp} = 0$, and $A_{\parallel} = 178 \text{ G}$. These parameters reveal that the interlayer Cu(II) ion forms a coordination group $[\text{Cu(II)(en)(OH}_2)_2]^{2+}$ (en; aminoethylamino group) and that the Cu(II) ion and the four coordinating atoms are almost in the same plane. Because the z axis of the principal system of g is perpendicular to the plane, the angular invariance indicates that the square plane is perpendicular to the silicate layers of montmorillonite.

We can assume, therefore, that pairs of CDen molecules with the coordination group directed inwards or outwards, form tail-to-tail or head-to-head dimers between the silicate layers, as illustrated in Fig. 3. In a tail-to-tail dimer (shown in more detail in Fig. 4), one of the two coordinating water molecules associated with one CDen molecule is likely to be hydrogen-bonded to one of the six primary hydroxyl groups in the other CDen molecule, resulting in the formation of double bridges between the two molecules. In a head-to-head dimer, on the other hand, the CDen molecules would associate face-to-face with hydrogen bonds between their secondary hydroxyl groups, as found in several cyclodextrin inclusion compounds². Moreover, these two types of dimers would be hydrogen-bonded through the secondary hydroxyl groups and coordinating water molecules, respectively, to the oxygens on the silicate surface. The bond angle and distance data for the glucose unit in CDen are given elsewhere^{9,10}. Considering the structures of several

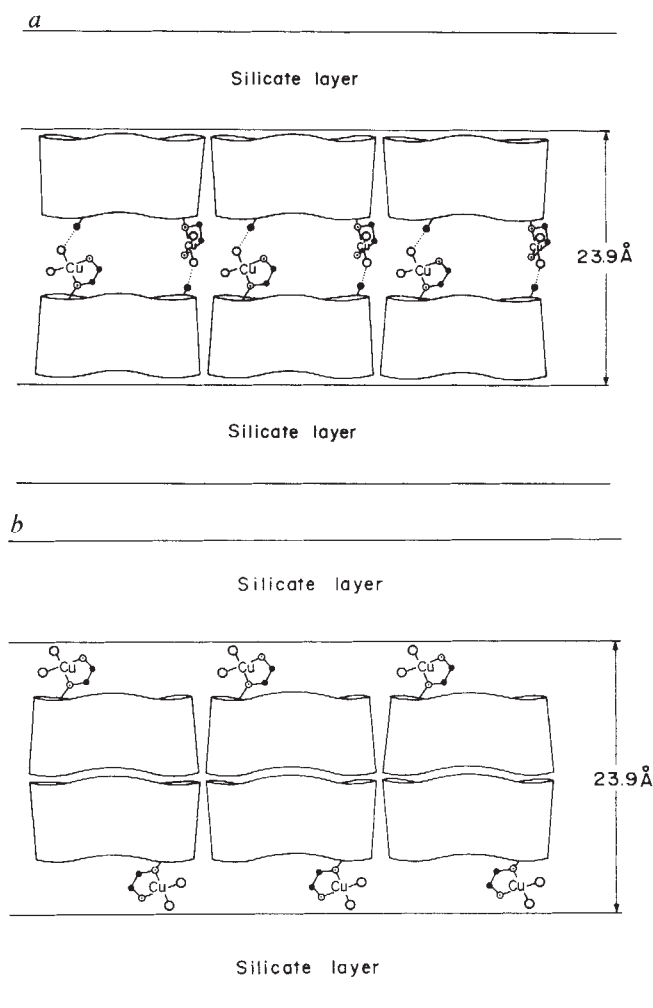


Fig. 3 Models proposed for the packing of CDen molecules in dimers with: *a*, tail-to-tail and *b*, head-to-head arrangements in the interlayer space of Cu(II)-montmorillonite. •, C; ○, N; ●, O; ○, water molecule. Hydrogen bonds are indicated by dotted lines.

cyclodextrin inclusion compounds^{2,9,10} and those of hydrates¹¹, it is reasonable to assume that the seven O(4) atoms are coplanar, that the pseudo-square plane composed of atoms C-1, C-2, C-5, and O-5 makes an angle of 80° with the heptagon, and that each length of the intermolecular hydrogen bonds in the two types of dimers is 2.8 Å. The structure data on Cu(II)(en)₂(NO₃)₂ (ref. 12) suggest that the Cu-N and Cu-O bonds are 2.0 Å in length. A value of 2.0 Å can be assumed for the van der Waals radii of the secondary hydroxyl group and coordinating water molecule located at both ends of a single CDen-Cu(II) complex¹³. The ESR result also means that the square plane of the coordination group in each dimer is perpendicular to the heptagonal plane of the parent molecule. Based on the above models, the van der Waals' thicknesses of the tail-to-tail and the head-to-head dimers were calculated to be 22.0 and 25.8 Å, respectively. The calculated value for the head-to-head dimer is also in good agreement with 13.7 × 2 minus 2.0 × 2 plus 2.8 or 26.2 Å, estimated semi-empirically using a value of 13.7 Å for the thickness of the monolayered CDen-Cu(II) complex, which was obtained for the CDen intercalate formed at the initial uptake stage and air-dried at 40 °C (ref. 6). Thus the most reliable value for the head-to-head dimer is 26.0 Å. On further heating for 15 h at 110 °C, the interlayer spacing of the monolayered phase remained unchanged, while the intercalated layer of the bilayered phase contracted from 23.9 to 22.9 Å, retaining the coordinating water molecules (according to ESR observations). The latter value is fairly close to the calculated value for the tail-to-tail dimer. The slight difference of 0.9 Å between the

observed and calculated values might be attributable to the deviation of the glucose units from their idealized positions due to the flexibility of the β-CDen macrocycle in each dimer. Thus, we suggest that CDen-Cu(II) complexes are more likely to be dimerized with tail-to-tail arrangement in the interlayer space; however, definite conclusions must await further work.

In view of the enzymatic function of CDen, the combined host compound corresponds to an immobilized enzyme and the intercalated layers of dimeric CDen-Cu(II) complexes may provide interesting models for membrane enzymes or lipid bilayer membranes. The complexation method developed in this study, in combination with the conventional methods², may afford an approach to improving the function of cyclodextrins. Many complexes of Cu(II)-, Ca-, K- or Na-montmorillonite with CDen or other cyclodextrin derivatives may be useful as artificial enzymes, solid supports in gas or liquid chromatography and as micro-encapsulating agents of unstable substances, especially insecticides, herbicides and soil disinfectants.

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1. Takemoto, K. in *Chemistry of Inclusion Compounds* Ch. 2 (Tokyo-Kagaku-Dojin, Tokyo, 1969).
2. Saenger, W. *Angew. Chem.* **19**, 344-362 (1980).
3. Bender, M. L. & Komiyama, M. *Cyclodextrin Chemistry* (Springer, Berlin, 1978).
4. Theng, B. K. G. in *The Chemistry of Clay-Organic Reactions*, Ch. 1 (Adam-Hilger, London, 1974).
5. Matsui, Y., Yokoi, T. & Mochida, K. *Chem. Lett.* 1037-1040 (1976).
6. Kijima, T., Kobayashi, M. & Matsui, Y. 3rd int. Symp. on Clathrate Compounds and Molecular Inclusion Phenomena—2nd int. Symp. on Cyclodextrins, Tokyo (1984).
7. Theng, B. K. G. in *The Chemistry of Clay-Organic Reactions*, 10 (Adam-Hilger, London, 1974).
8. Greenland, D. J., Laby, R. H. & Quirk, J. P. *Trans. Faraday Soc.* **58**, 829-841 (1962).
9. Hingerty, B. & Saenger, W. *J. Am. chem. Soc.* **98**, 3357-3365 (1976).
10. Fugiwara, T. *J. Cryst. Soc. Jap.* **24**, 54-64 (1982).
11. Wells, A. F. in *Structural Inorganic Chemistry* 4th edn, 301-304 (Clarendon, Oxford, 1975).
12. Wyckoff, R. W. G. in *Crystal Structures* 2nd edn Vol. 5, 344-345 (Interscience, New York, 1966).
13. Pauling, L. in *The Nature of Chemical Bond* 3rd edn, Ch. 7 (Cornell University Press, New York, 1960).

Two-dimensional chiral crystals of phospholipid

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Epifluorescence optical microscopy has been used to show the formation of solid phase domains from fluid phase domains on compression of dipalmitoylphosphatidylcholine (DPPC) monolayers at the air-water interface¹⁻⁴. These monolayers can be transferred to solid substrates⁴, and in certain conditions exhibit X-ray diffraction characteristic of highly ordered two-dimensional crystals⁵. In previous work, the crystal domains, visualized by the exclusion of fluorescent lipid probes, were often round and arrayed within a continuous fluid phase domain in a hexagonal pattern⁴. We report here that when DPPC monolayers are more rapidly compressed, at a rate of the order of a 2% decrease in area per second, chiral solid domains of lipid are formed. The handedness of the solid domains is directly related to the enantiomeric configuration of the lipids composing the monolayer. The shape of these domains provides direct visual evidence for long range orientational order in two-dimensional crystals.

Phosphatidylcholines possess a chiral centre at the C-2 carbon atom of the glycerol backbone. Cell membranes contain only one of the two enantiomers, which is the *R* isomer according to the *R/S* nomenclature system of Cahn *et al.*^{6,7}. Monolayers composed of either 2*R*,3-dipalmitoylglycerol-1-phosphorylcholine (*R*-DPPC, Sigma, 99%), 2*S*,3-dipalmitoylglycerol-1-phosphorylcholine (*S*-DPPC) or binary mixtures of the *R* and *S* isomers containing 2 mol % of the fluorescent lipid probe 3-acyl-2*R*-[*N*-(7-nitro-2,1,3-benzoxadiazol-

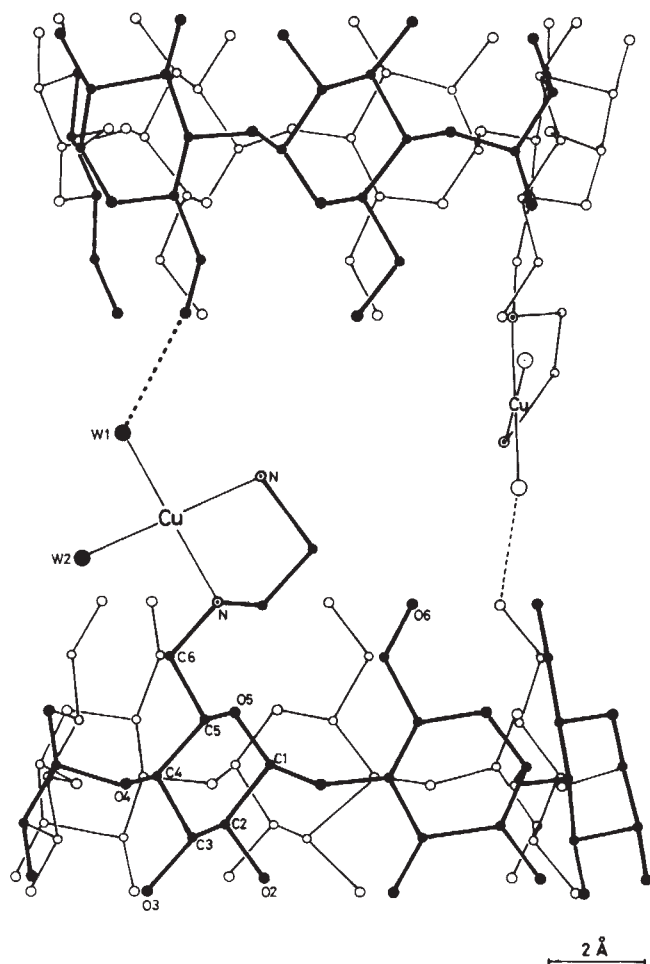


Fig. 4 Schematic diagram of the tail-to-tail dimer of 1:1 complex of Cu(II) with CDen; hydrogen atoms bonded to carbons are omitted. w1, w2, Water molecules. Hydrogen bonds are indicated by dashed lines. Parts of the CDen molecules closer to the viewer are heavily shaded.

4-yl)-aminocaproyl]-glycero-1-phosphorylcholine (NBD-PC, Avanti) were formed, observed and photographed at the air-water interface on the trough and Zeiss photomicroscope III system described earlier⁴. All of the domain structures reported here were observed at surface pressures and average molecular areas corresponding to the two-phase region of the pressure-area diagram for DPPC⁴. All monolayers were formed over a distilled water subphase and observed at ambient temperature ($22 \pm 2^\circ\text{C}$). In addition to a $\times 20$ objective, a long working distance $\times 40$ (Nikon) objective was also used. *S*-DPPC was separated from 3-palmitoyl-2*R*-glycero-1-phosphorylcholine and palmitic acid by silicic acid chromatography after treatment of *R,S*-DPPC (Sigma, 99%) with phospholipase A₂ (EC 3.1.1.4) from *Naja*

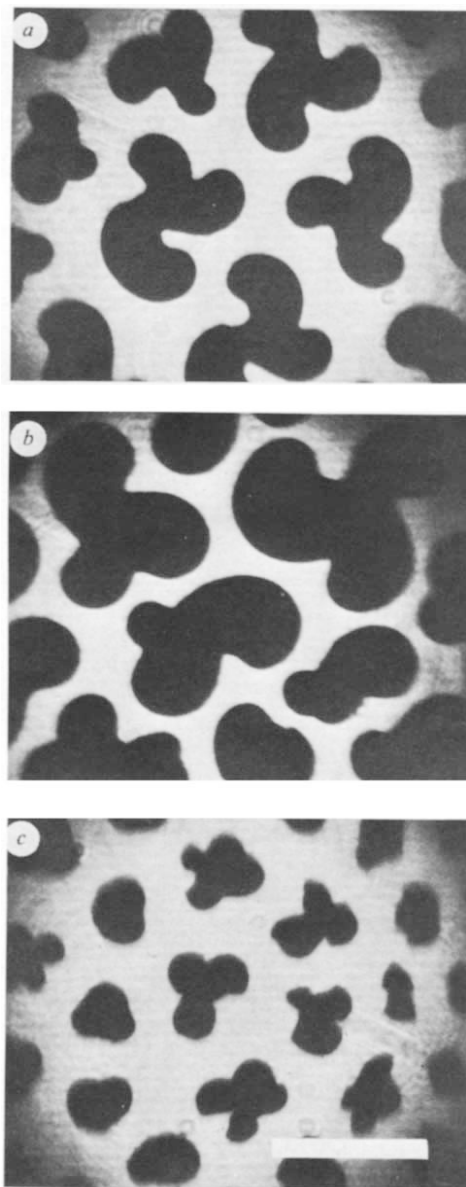


Fig. 1 Epifluorescence microscope photographs showing chiral solid domains in monolayers comprised of: *a*, 75% *R*-DPPC, 25% *S*-DPPC; *b*, 75% *S*-DPPC, 25% *R*-DPPC. No chirality is seen in the solid phase domains of racemic DPPC (equimolar *R*-DPPC and *S*-DPPC, *c*). The monolayers were formed over a distilled water subphase and observed at ambient temperature ($22 \pm 2^\circ\text{C}$). The monolayers contain 2 mol% of the fluorescent probe NBD-PC. Surface pressure and average molecular area for the monolayers are: *a*, 9 dyn cm^{-1} and $70 \text{ \AA}^2$; *b*, 7 dyn cm^{-1} and $68 \text{ \AA}^2$; *c*, 5 dyn cm^{-1} and $72 \text{ \AA}^2$, respectively. Similar solid domains to those shown in *a* and *b* were observed for monolayers composed of the respective pure enantiomorphs. Scale bar, $50 \mu\text{m}$.

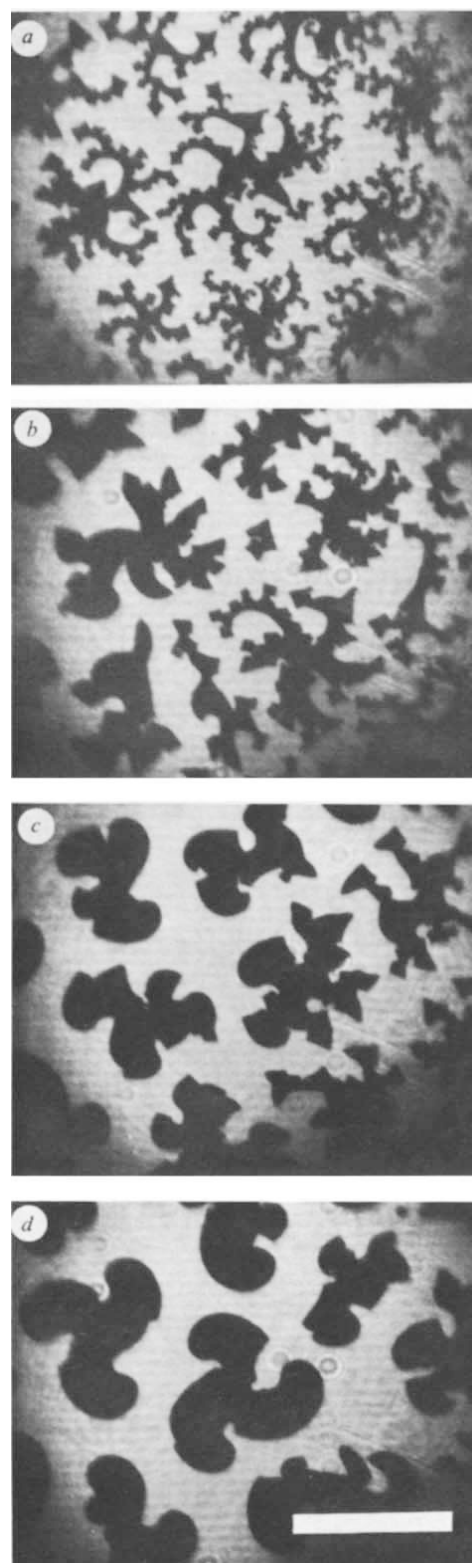


Fig. 2 Epifluorescence optical microscope photographs of chiral solid phase domains formed by non-uniform compression. *a-d*, Regions in the monolayer brought into view by slight membrane flow. The shape of the domains is due at least in part to different binding kinetics of fluid lipid to solid lipid on the different crystal faces. This is most dramatically seen in *a*. The monolayer is composed of 75% *R*-DPPC and 25% *S*-DPPC with 2 mol% of the total lipid as the fluorescent probe NBD-PC. The lateral pressure and the mean molecular area in the monolayer was 9 dyn cm^{-1} and $70 \text{ \AA}^2$, respectively. Fig. 1*a* would be the fifth photograph in this series. The subphase was distilled water and observations were made at ambient temperature ($22 \pm 2^\circ\text{C}$). Scale bar, $50 \mu\text{m}$.

naja (Sigma)^{8,9}. The *S*-DPPC was determined to have an enantiomeric excess >95% by polarimetry (Autopol III, Rudolph). Truly racemic *R,S*-DPPC was prepared from nominally racemic commercial DPPC by the addition of *S*-DPPC until a null optical rotation was obtained.

Figure 1*a, b* exhibit mirror-image solid phase domains formed from the binary mixtures of 75% *R*-DPPC, 25% *S*-DPPC and 75% *S*-DPPC, 25% *R*-DPPC, respectively. These domain structures are very similar to those formed by the corresponding pure enantiomorphs. The solid phase domains from the racemic mixture show no chirality (Fig. 1*c*).

The detailed shape of the solid phase domains is a sensitive function of the rate of compression (Fig. 2). Figure 2 represents a lateral variation of domain structure at the air-water interface due to non-uniform compression. (A wave of compression, abruptly launched at one end of a trough, is attenuated as it propagates in a two-phase region, giving rise to different rates of compression in the same monolayer.) Figure 2*a-d* shows adjacent regions of the monolayer brought into view by slight membrane flow, and illustrate some of the many crystal forms that appear on compression. At very low rates of compression, nearly round crystals are produced. At higher rates of compression, chiral forms with smooth edges are produced, most often with 3-fold rotation symmetry but sometimes also with 2-, 4- and 6-fold rotation symmetry. At still higher rates, complex chiral patterns are produced (see Fig. 2*a*). Aside from the effect of chirality, the domain structures formed are reminiscent of those observed in the formation of certain organic crystals from the melt at various degrees of supercooling¹⁰. The crystal shapes that we have observed do show two remarkable features. In some cases isolated solid phase domains form a regular pattern with both positional and orientational order of the domains (Fig. 3*a*). Also, some of the chiral forms develop an 'ingrown' spiral structure (Fig. 3*b*). These special features in Fig. 3 may involve overlap of the diffusion fields in the fluid lipid during crystal formation. This diffusion field is long-range in two dimensions. Electrostatic effects may also affect these structures. The solid phase domains formed at the air-water interface must have long-range molecular orientational order. This order is essential for the formation of chiral crystals. We cannot rule out the possibility of some twist of this orientational order if line tension leads to deformation of the crystals.

The solid domains formed by the racemic mixture of DPPC show no chirality. Thus these solid domains are either a compound, a eutectic, or a solid solution. Compound or eutectic formation is unlikely as careful pressure-area diagrams for the pure enantiomers and the racemic mixture are indistinguishable^{11,12}. (Enantiomeric discrimination is observed for monolayers of other substances at the air-water interface^{13,14}.) Also, the main chain-melting transition temperatures for bilayers of *R*-DPPC and *R,S*-DPPC are almost the same¹⁵. This suggests the solid (and liquid) solutions of *R*- and *S*-DPPC are nearly ideal (for electron diffraction studies of crystals of *R* and *R,S*-DPPC, see ref. 16). We have observed that a second fluorescent lipid probe, 2*R*,3-dipalmitoylglycero-1-phosphoryl-*N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-ethanolamine (Avanti), partitions strongly into both *R*- and *S*-DPPC crystals. This supports the idea of solid solutions.

It is surprising that such markedly chiral solid domains should be formed by solid solutions which may be close to ideal. There must be marked differences in the kinetics of fluid lipid → solid lipid binding on the different crystal surfaces, as shown: especially in Fig. 2*a*. Such differences might arise, for example, if the solid phase monolayer had tilted chains and was ferroelectric. Reported crystal structures suggest this possibility^{17,18}. Deliberately added impurities, such as fatty acids, affect the sizes of the solid phase domains but do not change the results qualitatively. Also, the partition coefficients of various fluorescent probes between the solid and fluid phases appear to affect crystal form (data not shown).

It is reasonable to hope that the general theoretical methods used by Langer¹⁹ for the morphology of snowflakes²⁰ can be

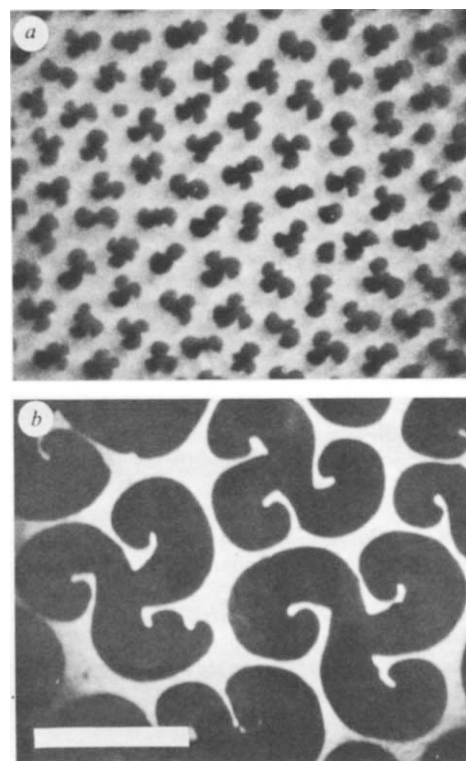


Fig. 3 Epifluorescence microscope photographs of chiral solid phase domains in monolayers composed of *R*-DPPC and 2 mol % NBD-PC. *a*, The similarity of shape of the domains and the positional and orientational order that is often observed between domains. *b*, A chiral domain that has grown back on itself to form an inwardly growing spiral. The conditions of surface pressure and average molecular area were: *a*, 9 dyn cm⁻¹ and 65 Å²; *b*, 9 dyn cm⁻¹ and 50 Å², respectively. Scale bar in *b*, 50 μm. Magnification in *a* is twice that of *b*. The crystals in *a* and *b* are formed from the same phospholipid enantiomer. Our experience is that crystals of the size and general shape as those in *a* or *b* would each have opposite handedness when formed from the other enantiomer, except for second-order effects related to the chirality and partition coefficient of the fluorescent probe used.

used to understand the observed structures. These structures may also be useful for studies of immune attack on supported monolayer membranes²¹.

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1. Losche, M., Sackmann, E. & Mohwald, H. *Ber. Bunsenges. phys. Chem.* **87**, 848-852 (1983).
2. Peters, R. & Beck, K. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7183-7187 (1983).
3. Beck, K. thesis, Univ. Frankfurt (1984).
4. McConnell, H. M., Tamm, L. K. & Weis, R. M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3249-3253 (1984).
5. Seul, M., Eisenberger, P. & McConnell, H. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5795-5797 (1983).
6. Cahn, R. S., Ingold, C. K. & Prelog, V. *Angew. Chem.* **78**, 413-447 (1966).
7. Hauser, H., Pascher, I., Pearson, R. H. & Sundell, S. *Biochim. biophys. Acta* **650**, 21-51 (1981).
8. Van Deenen, L. M. M. & De Haas, G. H. *Biochim. biophys. Acta* **70**, 538-553 (1963).
9. Bergelson, L. D. *Lipid Biochemical Preparations*, 133-139 (Elsevier, Amsterdam, 1980).
10. Ovsienko, D. E. & Alfintsev, G. A. in *Crystals, Growth and Properties* Vol. 2, 120-165 (Springer, Heidelberg, 1980).
11. Phillips, M. C. & Chapman, D. *Biochim. biophys. Acta* **163**, 301-313 (1968).
12. Arnett, E. M. & Gold, J. M. *J. Am. chem. Soc.* **104**, 636-639 (1982).
13. Cadenhead, D. A. *Rev. Prog. Surf. Sci.* **3**, 169-192 (1970).
14. Stewart, M. V. & Arnett, E. M. *Topics Stereochem.* **13**, 195-262 (1981).
15. Boyanov, A. I., Tenchov, B. G., Koynova, D. & Koumanov, K. S. *Biochim. biophys. Acta* **732**, 711-713 (1983).
16. Sakurai, I. et al. *Chem. Phys. Lipids* **26**, 41-48 (1980).
17. Ruocco, M. J. & Shipley, G. *Biochim. biophys. Acta* **691**, 309-320 (1982).
18. Pearson, R. H. & Pascher, I. *Nature* **281**, 499-501 (1979).
19. Langer, J. S. *Rev. mod. Phys.* **52**, 1-28 (1980).
20. Maddox, J. *Nature* **306**, 13 (1983).
21. Weis, R. M., Balakrishnan, K., Smith, B. A. & McConnell, H. M. *J. biol. Chem.* **257**, 6440-6445 (1982).

A high rate of self-fertilization and increased seed fertility of homostyle primroses

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There has been extensive theoretical study of the evolution of self-fertilization in hermaphroditic or monoecious flowering plants because self-compatible species with high rates of self-fertilization are often derived from outcrossing ancestors¹⁻⁴. Such studies have shown that a gene causing an increased rate of self-fertilization has an advantage because the mother transmits genes via both pollen and egg to selfed progeny⁵, and thereby evades the 'cost of meiosis'⁶⁻⁸. Selfing also may be advantageous if the amount of pollen available to fertilize ovules by outcrossing is limited^{1,2,4,6}. However, these advantages are offset, at least partially, if the fitness of selfed progeny is lower than that of outcrossed progeny, due to inbreeding depression^{1,2,6,7,9,10}. Biogeographical evidence suggests that reduced pollinator activity may be sufficient to counteract this disadvantage and permit the evolution of selfing in populations in ecologically marginal areas¹⁻⁴, but direct evidence is usually lacking. Here we show that the self-fertile homostyle variant of the primrose *Primula vulgaris*, which reaches high frequencies in certain populations¹¹, experiences a fertility advantage, in terms of seeds per plant, as a result of its high rate of self-fertilization.

Populations of *P. vulgaris* are usually composed of the heterostyled pin and thrum forms, in approximately equal frequencies¹⁰. These are morphologically distinct (pin having a long style and low anthers, and thrum the reverse) and are self-incompatible but cross-compatible^{10,11}. The long homostyle variant is self-fertile, and combines the pin female characters with the thrum male characters so that the anthers normally surround the stigma, and is inherited as an allele at the heterostyly locus¹¹. The locally high frequencies of the homo-

style variant in populations in Somerset, S.W. England, have been investigated for several years by Crosby^{11,12} and, more recently, by C. F. and J. Curtis (unpublished results). The extent to which the homostyles self-fertilize has been a matter of controversy^{12,13}. First, we provide evidence that the homostyles have a high rate of selfing: this is clearly crucial in testing models of their spread^{1,11,13}.

The population from Wyke Champflower was chosen for study. It consists of approximately 50% pin, 25% thrum and 25% homostyle plants. Seed capsules were collected in spring 1982, and seeds were raised in family batches from 10 homostyle, 7 pin and 4 thrum mother plants. In order to estimate selfing rates, polyacrylamide gel electrophoresis was performed on 20 seedlings from each homostyle family, and 9-14 from the pin and thrum families. Two polymorphic, diallelic enzyme loci, *Est* and *Acph* (which control an α -esterase and an acid phosphatase, respectively), were scored using the methods of Piper (in preparation). Once the genotype of each plant had been established with certainty, a modification of the maximum likelihood procedure of Brown *et al.*¹⁴ was used to estimate maternal genotype, pollen gene frequency, and the rate of self-fertilization (*s*) for each locus separately. The homostyles had *s* values ($\pm$ s.e.) of 0.92 ± 0.09 and 0.92 ± 0.09 using *Est* and *Acph* respectively; neither of these values differs significantly from unity. In contrast, the pooled *s* value for pin and thrum was -0.11 ± 0.15 for *Est*, which is significantly lower than the corresponding homostyle values ($P < 0.01$) but is not significantly different from zero. There may be an upward bias in the estimates of *s* due to population sub-structuring¹⁵, but this is unlikely to be important in this species (C. M. Cahalan, in preparation). The estimates of *s* for the homostyles are close to the value estimated by Crosby¹², using segregation at the heterostyly locus in plants from natural populations.

The high selfing rate of the homostyles can be explained by comparing the compatible pollen loads on pin and homostyle stigmas. This can be done because pollen grains from thrum-type anthers (characteristic of both thrum and homostyle plants) are much larger than those from pin anthers, and are easily distinguishable microscopically¹⁰. Large thrum-type pollen on pin stigmas represents the inter-plant movement of pollen from

Table 1 Fertility data for the three morphs of *Primula vulgaris* from seven populations

1982	P	T	H	P	T	H	P	T	H	P	T	H	P	T	H
Morph frequency	0.50	0.50	—	0.45	0.45	0.10	0.50	0.25	0.25	0.19	0.35	0.46	0.20	—	0.80
Whole plant fertility	P 480 $\pm$ 60			647 $\pm$ 122			213 $\pm$ 21			316 $\pm$ 58			315 $\pm$ 46		
	T 475 $\pm$ 50			777 $\pm$ 113			238 $\pm$ 33			143 $\pm$ 21			—		
	H —			805 $\pm$ 142			255 $\pm$ 29			513 $\pm$ 49†			408 $\pm$ 54		
Seeds per capsule	P 49.2 $\pm$ 3.2			45.4 $\pm$ 3.6			42.2 $\pm$ 1.8			35.6 $\pm$ 4.1			44.3 $\pm$ 2.9		
(natural pollination)	T 43.2 $\pm$ 2.5			47.0 $\pm$ 4.0			44.1 $\pm$ 2.3			23.2 $\pm$ 3.4			—		
	H —			58.7 $\pm$ 2.5†			52.3 $\pm$ 2.3*			45.0 $\pm$ 2.0†			46.1 $\pm$ 1.7		
Seeds per capsule	P 47.9 $\pm$ 3.1			44.9 $\pm$ 4.1			56.7 $\pm$ 4.7‡			42.7 $\pm$ 5.4§			56.6 $\pm$ 3.6‡		
(artificial pollination)	T 44.1 $\pm$ 2.9			47.5 $\pm$ 3.6			55.7 $\pm$ 3.9‡			42.1 $\pm$ 5.3§			—		
1983	P	T	H	P	T	H	P	T	H	P	T	H	P	T	H
Morph frequency	0.50	0.50	—	0.46	0.42	0.12	0.50	0.25	0.25	0.41	0.13	0.46	0.20	—	0.80
Whole plant fertility	P 154 $\pm$ 41			112 $\pm$ 28			144 $\pm$ 30			163 $\pm$ 36			146 $\pm$ 22		
	T 86 $\pm$ 25			40 $\pm$ 19			144 $\pm$ 26			19 $\pm$ 8			—		
	H —			316 $\pm$ 53†			364 $\pm$ 45†			374 $\pm$ 47†			201 $\pm$ 33		
Seeds per capsule	P 18.3 $\pm$ 3.5			12.6 $\pm$ 2.5			24.2 $\pm$ 3.3			18.3 $\pm$ 2.9			27.2 $\pm$ 3.3		
(natural pollination)	T 19.1 $\pm$ 4.8			8.9 $\pm$ 3.6			23.9 $\pm$ 3.0			3.5 $\pm$ 2.4			—		
	H —			48.9 $\pm$ 3.7†			43.1 $\pm$ 2.3†			42.9 $\pm$ 3.3†			34.7 $\pm$ 2.6*		
Seeds per capsule	P 44.9 $\pm$ 4.8§			47.4 $\pm$ 4.9§			49.1 $\pm$ 4.1§			46.4 $\pm$ 4.3§			49.4 $\pm$ 9.9§		
(artificial pollination)	T 52.0 $\pm$ 5.9§			35.8 $\pm$ 7.3§			45.8 $\pm$ 5.1§			44.5 $\pm$ 4.9§			—		

Fertility data of pin (P), thrum (T) and homostyle (H) plants at seven sites, three of which (the 0%, 10% and 80% homostyles sites) were studied in 1982 and 1983. In both seasons it was common for homostyles to produce more seeds per capsule than pins and thrums, though only in the second season was it common for homostyle plants to produce more seeds than pin and thrum plants (homostyles produced significantly more seeds per capsule/plant than pins and thrums: * $P < 0.05$, † $P < 0.01$). In all but two sites (0% and 10% homostyle sites in 1982) the differences in fertility observed were attributable to compatible pollen load size (compatibly pollinated pin and thrum plants produced more seeds per capsule than naturally pollinated ones: ‡ $P < 0.05$, § $P < 0.01$). Sample sizes for each morph varied from 15 to 50, depending on the frequency of each morph and the size of the population.

thrum and homostyle plants, whereas thrum-type pollen on homostyle stigmas represents both intraplant (selfing) and interplant (outcrossing) pollen. The pin pollen may be disregarded as it is incompatible. The selfing component of pollen load on homostyle stigmas can thus be obtained by subtracting the mean thrum-type pollen load on pin stigmas from the mean for homostyles. Such an experiment was performed on a population at Wanstrow, which has pin, thrum and homostyles in frequencies of 0.45, 0.45 and 0.10 respectively, so that pin- and thrum-type stigmas and anthers are approximately equally frequent. Stigmas from 30 pin and 20 homostyle flowers were collected from separate plants at the height of the 1982 flowering season. They were fixed in a 1:1 mixture of absolute ethanol and glacial acetic acid, after which they were acetolysed¹⁶ individually, and the number of thrum-type pollen grains counted for each stigma. The mean number of thrum-type pollen grains per stigma was 33 ± 18.5 for pin and $1,217 \pm 198.0$ for homostyle. The estimate for pin stigmas agrees closely with that obtained by Cahalan (in preparation) for a North Wales population, and by Ornduff¹⁷ for an English population. The mean number of self-produced pollen grains on homostyle stigmas is thus $1,184 \pm 190$, representing 97% of the compatible pollen load. The high selfing rate of the homostyles is therefore not surprising.

The higher compatible pollen load on the homostyle stigmas, compared with pins, suggests that the homostyle plants might well have higher seed fertilities than the self-incompatible morphs, unless seed production is strictly resource-limited¹⁸ in these populations. This was tested in both the 1982 and 1983 flowering seasons in several Somerset populations (see Table 1). A large sample of plants from each population was followed throughout the flowering season in each year. The number of capsules that set seed and survived predation was recorded for each plant. At the end of the season (late May/early June), several capsules were collected from each plant, and the numbers of seeds per capsule were counted. Combining this with the information on the numbers of fruiting capsules per plant, the total seed output for each plant can be estimated, and the mean seed output per plant calculated for each morph (Table 1). In 1982, homostyles produced more seed per plant than the other two morphs in each population, although the differences were not always significant. In the 1983 season, homostyles produced significantly more seed at all but one site, where the pins (thrums were absent) produced 50% more flowers than the homostyles.

It could be argued that the superior seed production of the homostyles is due to the expected reallocation of resources from male to female reproduction in a highly selfing form^{6,19,20}, rather than to their higher compatible pollen loads. To test this hypothesis, one flower of each pin and thrum plant studied was pollinated with compatible pollen by hand at each site in both seasons. At the end of the season, the numbers of seeds per capsule were determined. As shown in Table 1, there is usually a large increase in the number of seeds per capsule of the hand-pollinated pin and thrum plants, making them as fertile as the homostyle capsules. This strongly supports the hypothesis that the lower seed fertilities of pin and thrum are due to lower compatible pollen loads. In 1982, there were two sites where hand pollination failed to increase seed set, suggesting that pollinator activity varies from place to place in some years at least. But in 1983, hand pollination always increased seed set significantly; this is correlated with very wet weather during the flowering season (information provided by Wessex Water Authority), which presumably reduced pollinator activity. Further evidence against the resource allocation hypothesis comes from two sources. First, measurements of male and female investment in ovules and pollen do not differ significantly between the three morphs (unpublished results). Second, the amount of compatible pollen on pin stigmas is insufficient to fertilize all the ovules, of which there are 50–60 (ref. 17).

We conclude that increased seed set of the homostyle morph, compared with the self-incompatible pin and thrum morphs, is due to its high rate of self-fertilization, which may be attributed to efficient self-pollination caused by the homostyle floral struc-

ture. This higher relative fertility of the homostyle morph may account, at least in part, for its spread in Somerset populations of *P. vulgaris*. Although a paucity of pollinators may not be responsible for the increase of homostyles in this case, the fertility data, particularly those for the wetter 1983 season, indicate how this factor may be relevant to the evolution of self-compatibility in habitats where conditions are inhospitable to pollinators^{1,2,4,6}. The fluctuations observed in the relative fertilities of the morphs between 1982 and 1983 show that only long-term studies of fitness components can provide conclusive evidence about the net direction of selection, and the reasons for the establishment of homostyles in such a small area.

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1. Charlesworth, B. & Charlesworth, D. *Am. Nat.* **114**, 499–513 (1979).
2. Lloyd, D. G. in *Demography and Evolution in Plant Populations* (ed. Solbrig, O. T.) 67–88 (Blackwell, Oxford, 1980).
3. Ernst, A. *Oesterr. Bot.* **2**, 100, 235–255 (1953).
4. Baker, H. G. *Evolution* **20**, 349–368 (1966).
5. Fisher, R. A. *Ann. Eugen.* **12**, 169–171 (1941).
6. Maynard Smith, J. *The Evolution of Sex* (Cambridge University Press, 1978).
7. Charlesworth, B. *J. theor. Biol.* **84**, 655–671 (1980).
8. Williams, G. C. *Sex and Evolution* (Princeton University Press, 1975).
9. Lande, R. & Schemske, D. W. *Evolution* (in the press).
10. Darwin, C. R. *The Different Forms of Flowers on Plants of the Same Species* (Murray, London, 1877).
11. Crosby, J. L. *Evolution* **3**, 212–230 (1949).
12. Crosby, J. L. *Heredity* **13**, 127–131 (1959).
13. Bodmer, W. F. *Phil. Trans. R. Soc. B* **242**, 517–549 (1960).
14. Brown, A. H. D., Matheson, A. C. & Eldridge, K. G. *Aust. J. Bot.* **23**, 931–939 (1975).
15. Ennos, R. A. & Clegg, M. T. *Heredity* **48**, 283–292 (1982).
16. Erdtman, G. *Handbook of Palynology* (Munksgaard, Copenhagen, 1969).
17. Ornduff, R. *Biol. J. Linn. Soc. Lond.* **78**, 1–10 (1979).
18. Stephenson, A. G. *A. Rev. Ecol. Syst.* **12**, 253–280 (1981).
19. Charlesworth, D. & Charlesworth, B. *Biol. J. Linn. Soc. Lond.* **15**, 57–74 (1981).
20. Charnov, E. L. *The Theory of Sex Allocation* (Princeton University Press, 1982).

Brain grafts can restore irradiation-damaged neuronal connections in newborn rats

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Immature rat brain tissue grafted to the brain of other immature and adult rats can survive and establish nerve connections with the host brains^{1–5}. In addition to facilitating the study of factors involved in the formation of central neural connections, brain grafts may also be used to substitute damaged or maldeveloped neurones^{5–10}. With exceptions in the visual system⁵, the restoration of specific central neural connections has to date involved grafts of cholinergic and monoaminergic neurones, which have good regenerative capacity¹⁰. In the present study, rat hippocampal neurones were damaged by neonatal X-ray irradiation and replaced by transplantation of normal, developing neurones of the same type. The grafted neurones (dentate granule cells) are not cholinergic or monoaminergic, but when appropriately located in the host hippocampal region they established specific and highly ordered afferent and efferent connections with the damaged host brain. Moreover, simultaneous demonstration of afferent and efferent transplant pathways showed that serial host–transplant–host connections had formed, restoring the normal neuronal circuitry initially disrupted by the irradiation.

The granule cells of the dentate gyrus assume a key position in the neuronal circuitry of the hippocampal region. Their dendrites receive in a precisely laminated fashion three major extrinsic inputs: the lateral and medial perforant pathways from the entorhinal cortex^{11–13} and the mixed commissural–associational projection from the dentate hilus^{14,15} (Fig. 1a,b). The granule cell axons, or mossy fibres, in turn terminate in the dentate hilus and in a distinct band along the hippocampal CA3

Fig. 1 Neuronal connections of rat dentate gyrus shown schematically (*a*) and by the histochemical Timm method (*b*). Lateral (lpp) and medial (mpp) perforant pathways from the entorhinal cortex terminate separately along the distal parts of the granule cell dendrites with commissural (c) and associational (a) projections overlapping on the proximal dendrites. The granule cell axons, or mossy fibres (mf), project to the dentate hilus (h) and in a narrow band along the hippocampal CA3 pyramidal cell layer (p). They do not enter CA1. *b*, *c*, Timm-stained section from adult rat X-irradiated at birth. The reduction of dentate granule cells has resulted in subnormal numbers of mossy fibres (mf in *b*) and aberrant perforant path projections to CA3 (asterisk). $\times 23$.

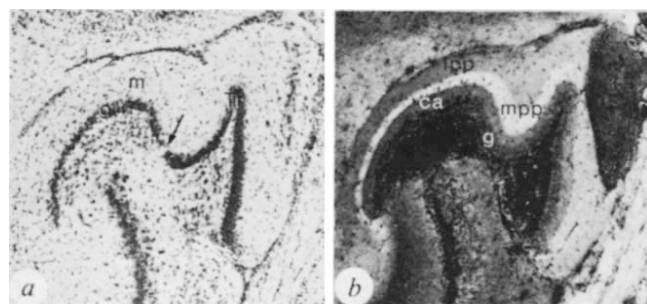
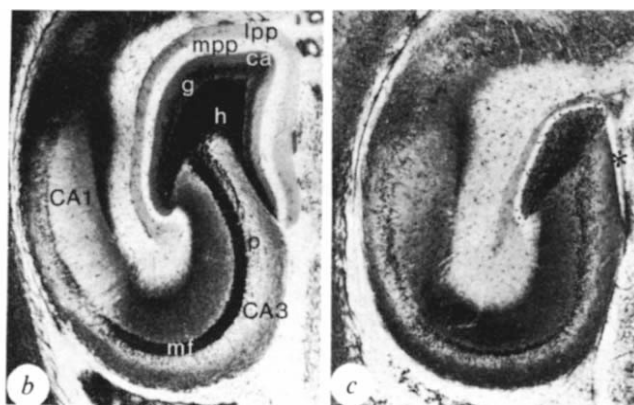
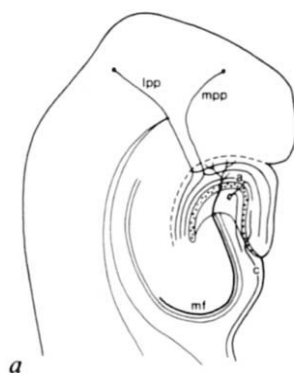


Fig. 2 *a*, Dentate transplant (Tr) encroaching on the irradiated host dentate gyrus. The granule cell (g) and molecular (m) layers of the two have fused (arrow at transition). Donor, normal newborn (PO); recipient, X-irradiated newborn; survival $3\frac{1}{2}$ months. Thionine cell staining. *b*, Adjacent Timm-stained section showing continuity between host and transplant of the laminar perforant path and commissural-associational terminal fields. $\times 26$.

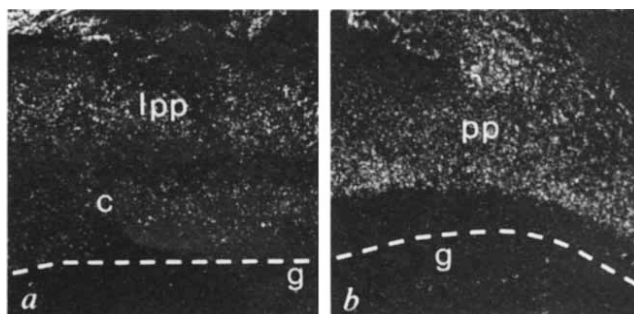


Fig. 3 Dentate transplants receiving specific, laminar host projections demonstrated in dark field after silver staining of anterograde degeneration. *a*, Laminar terminations of host commissural (c) and lateral perforant path (lpp) projections spaced around untraced medial perforant path in transplant molecular layer. *b*, Laminar termination of host lateral and medial perforant pathways (pp) in outer parts of transplant molecular layer. In both cases: donor, newborn rat; recipient, X-irradiated newborn rat; survival 4 months. Broken line, superficial border of the granule cell layer (g). $\times 108$.

pyramidal cell layer^{16,17}, as demonstrated by the Timm silver-sulphide method¹⁸ which also shows the afferent terminal zones in the dentate molecular layer¹⁹ (Fig. 1*b*). More than 80% of the rat dentate granule cells form after birth, when all other hippocampal and entorhinal neurones are present²⁰. Postnatal X-irradiation can therefore almost selectively reduce the granule cell population to about 15% of normal²¹. In these conditions granule cell afferents, now in 'excess', form aberrant but 'appropriately' laminated projections to the CA3 pyramidal cells, while the mossy fibre projection is severely reduced²² (Fig. 1*c*). The situation after neonatal X-ray irradiation in many respects mimics naturally occurring maldevelopments or early degenerative diseases. With this in mind we wished to examine whether the damaged dentate granule cells could be substituted by transplanted normal ones.

Rat pups were X-irradiated bilaterally (600 rad) over the hippocampal region on the day of birth²², after which normal dentate tissue from other newborn rats was grafted into the hippocampal region on one side⁴. After $3\frac{1}{2}$ –5 months survival the animals were killed and parallel series of brain sections processed with the Timm method for the demonstration of mossy fibre terminals and terminal fields in the dentate molecular layer¹⁹, acetylcholine esterase staining for cholinergic projections²³ and thionine cell staining. Most animals in addition received entorhinal or contralateral hippocampal lesions before death, in order to trace host connections into the transplants by axonal degeneration methods.

Specific laminar innervation of dentate transplants by host perforant path and commissural fibres were observed when the transplants encroached on and fused with the irradiated host

dentate gyrus (Figs 2*a*, 3*a*). Appropriately-terminating host perforant path afferents were also traced into transplants located along the route of this pathway (Fig. 3*b*), but no commissural input was traced to these transplants, presumably because of the distance to the normal commissural trajectory.

From dentate transplants that encroached on the host CA3 region, Timm-stained mossy fibre terminals passed into the adjacent host mossy fibre layer (Fig. 4). The almost normal amounts of terminals here were in marked contrast to the X-ray-induced paucity of terminals some levels away on the same side and on the entire contralateral non-transplanted side which served as control (compare Fig. 4*a* and *b*). Although arriving from abnormal directions, most transplant-related terminals were confined to the normal mossy fibre zone along the apical part of the CA3 pyramidal cell layer (Fig. 4*a*). Within this zone the terminals were primarily distributed in the normal direction towards the host CA1 area, and like normal mossy fibres they stopped at the CA3–CA1 transition.

In 4 of 38 animals studied there were specific hosts–transplant–host connections with host perforant path and commissural projections to dentate transplants which in turn sent mossy fibres to the host CA3. The transplants were in these cases situated immediately next to or in the irradiated host dentate gyrus, stressing the importance of transplant location for appropriate exchange of connections.

'Misplacement' of transplants could result in the formation of abnormal serial connections. The transplant, shown in Fig. 4*a* to send mossy fibres to the host CA3, thus received afferents from the contralateral hippocampus with an abnormal termination within its molecular layer. Previous studies have shown that

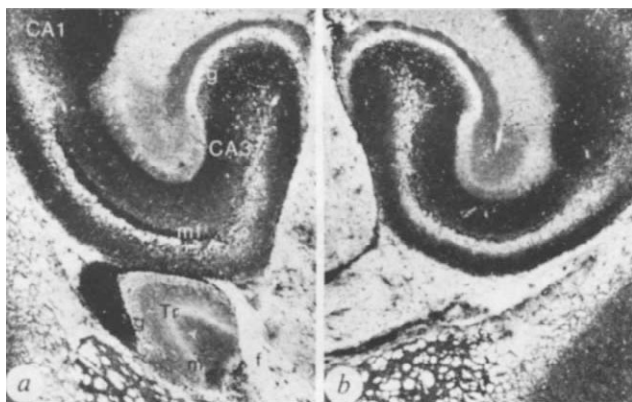


Fig. 4 Transplanted (a) and control side (b) of irradiated rat, with ingrowth of Timm-stained mossy fibres (mf) from dentate transplanted (Tr) into the specific target zone of the host CA3. The transplanted received correct septal but non-specific commissural host afferents in its molecular layer (m). Donor, newborn rat; recipient, X-irradiated newborn rat; survival 4 months. $\times 23$.

these afferents are likely to originate from parts of the hippocampus not normally projecting to the dentate gyrus⁴.

For the ingrowth of host cholinergic fibres, the site of the transplanted was less critical, as judged by the presence of acetylcholine esterase staining in all transplants. Through this ingrowth, serial septal–dentate–hippocampal connections were formed in all transplants, sending mossy fibres into the host CA3.

The demonstration of specific and topographically organized host–transplant interconnections is new for hippocampal transplants and almost unique for non-cholinergic and non-monoaminergic systems. It raises not only important reparative, but also developmental considerations. At the time of X-irradiation and transplantation, few fibres of the extrinsic and intrinsic hippocampal fibre systems have reached their terminal areas^{19,24,25}. The normal laminar segregation of host perforant path and commissural–associational afferents in the transplant molecular layer accordingly developed after transplantation, and most probably later than normal, allowing for the transplanted tissue to become incorporated in the host brain. Precise timing²⁶ may thus not be critical for a proper segregation of afferents within the dentate gyrus. Transplant mossy fibres to the host were undoubtedly similarly delayed in growth, but they also formed appropriate connections, even when they entered along unusual routes related to the position of the transplant. This specificity in termination is in contrast to previous reports where dentate transplants were positioned within the CA1 region of developing²⁷ and adult rats²⁸.

The present study gives the first demonstration that an irradiation-induced loss of nerve cells in the developing rat brain can at least in part be replaced by the transplantation of normal immature neurones of the same type. Future studies will examine whether the structural repair of the damaged neuronal circuitry may also result in functional compensation of behavioural deficits observed after X-irradiation²⁹. Additionally, we are currently examining the effects of delaying the transplantation relative to the time of X-irradiation.

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- Lund, R. D. & Hauschka, S. D. *Science* **193**, 582–584 (1976).
- Björklund, A. & Stenevi, U. *Cell Tissue Res.* **186**, 289–302 (1977).
- Oblinger, M. M., Hallas, B. H. & Das, G. D. *Brain Res.* **189**, 228–232 (1980).
- Sunde, N. A. & Zimmer, J. *Devl Brain Res.* **8**, 165–191 (1983).
- Lund, R. D. & McLoon, S. C. in *Neural Tissue Transplantation Research* (eds Waller, R. B. & Das, G. D.) 165–174 (Springer, New York, 1981).
- Björklund, A. & Stenevi, U. *Brain Res.* **177**, 555–560 (1979).
- Gash, D., Sladek, J. R. Jr & Sladek, C. D. *Science* **210**, 1367–1369 (1980).
- Krieger, D. T. *et al. Nature* **298**, 468–471 (1982).
- Low, W. C. *et al. Nature* **300**, 260–262 (1982).
- Björklund, A. *et al. Acta physiol. scand. Suppl.* **522**, 1–75 (1983).

- Hjorth-Simonsen, A. & Jeune, B. *J. comp. Neurol.* **144**, 215–232 (1972).
- Hjorth-Simonsen, A. *J. comp. Neurol.* **146**, 219–232 (1972).
- Steward, O. *J. comp. Neurol.* **167**, 285–314 (1976).
- Zimmer, J. *J. comp. Neurol.* **142**, 393–416 (1971).
- Laurberg, S. *J. comp. Neurol.* **184**, 685–708 (1979).
- Blackstad, T. W., Brink, K., Hem, J. & Jeune, B. *J. comp. Neurol.* **138**, 433–450 (1970).
- Gaarskjaer, F. B. *J. comp. Neurol.* **203**, 717–735 (1981).
- Haug, F.-M. S., Blackstad, T. W., Simonsen, A. H. & Zimmer, J. *J. comp. Neurol.* **142**, 23–32 (1971).
- Zimmer, J. *Prog. Brain Res.* **48**, 171–189 (1978).
- Bayer, S. A. *J. comp. Neurol.* **190**, 87–114 (1980).
- Bayer, S. A. & Altman, J. *J. comp. Neurol.* **163**, 1–20 (1975).
- Laurberg, S. & Hjorth-Simonsen, A. *Nature* **269**, 158–160 (1977).
- Geneser-Jensen, F. A. & Blackstad, T. W. *Zellforsch.* **115**, 460–481 (1971).
- Cowan, W. M., Stanfield, B. B. & Amaral, D. G. in *Studies in Developmental Neurobiology* (ed. Cowan, W. M.) 395–435 (Oxford University Press, 1981).
- Stirling, R. V. & Bliss, T. V. P. *Prog. Brain Res.* **48**, 191–198 (1978).
- Gottlieb, D. I. & Cowan, W. M. *Brain Res.* **41**, 452–456 (1972).
- Sunde, N. & Zimmer, J. *Neurosci. Lett. Suppl.* **7**, S33 (1981).
- Raisman, G. & Ebner, F. F. *Neuroscience* **9**, 783–801 (1983).
- Bayer, S. A., Brunner, R. L., Heni, R. & Altman, J. *Nature* **242**, 222–224 (1973).

Staining of living presynaptic nerve terminals with selective fluorescent dyes

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Johnson *et al.* have recently shown that several positively charged, membrane-permeant fluorescent dyes can serve as vital stains for mitochondria in cultured cells^{1,2} (see also ref. 3). We report here that presynaptic nerve terminals, which are characteristically rich in mitochondria^{4,5}, can also be vitally stained with such dyes, and that this staining offers a resolution of structural detail not available with previous methods for visualizing terminals in living tissues. The dyes have provided excellent, highly detailed fluorescence images of presynaptic motor nerve terminals at neuromuscular junctions in conventional live tissue preparations from mouse, frog and *Drosophila*. In addition, when tested at the frog neuromuscular junction, at least one of the dyes permitted visualization of terminals with little, if any, effect on synaptic transmission. Moreover, we have found that by using the dyes it is now also possible to see motor nerve terminals *in situ* in live animals.

Figures 1 and 2 show examples of endplates in living, isolated preparations of skeletal muscles from frog⁶ and mouse⁷ stained with the dye 3,3'-diethyloxadycarbocyanine iodide [DiOC₂(5)]. The presynaptic nerve terminals appear brightly fluorescent and sharply defined against background muscle fibres, which are also labelled. The appearance of the label in muscle fibres varied with the source of the preparation. In frog muscles, the fluorescence, presumably in mitochondria, appeared as long filaments parallel to the fibre axis, as coarse grains, or sometimes as bow tie-shaped particles. Fluorescence intensity was often correlated with the sarcomere pattern visible with bright-field optics, and in mouse muscle fibres this variation was the predominant feature, with particles being less evident. Generally, little or no fluorescence was seen in other structures such as the preterminal nerve, myelin, blood vessels, red blood cells or connective tissue.

The fidelity of nerve terminal labelling was assessed by comparing photomicrographs of living endplates stained with DiOC₂(5) with the same endplates after fixation and staining by the zinc iodide–osmium (ZIO) procedure, which selectively blackens nerve terminals^{6,8}. The correspondence of terminal morphology revealed by the two stains was exact to a very fine level (see Fig. 2). In frog preparations, for example, both stains revealed identical periodicities occurring along the terminals, giving them a striated or beaded appearance (Fig. 2a, a'). Such periodicities have been shown by electron microscopy to be a consequence of crenellations in the terminals produced by finger-like projections of Schwann cell inserted between the nerve terminal and muscle⁶, and have not previously been visible in living terminals, viewed for example by differential interference contrast (DIC) optics. The exact agreement between the

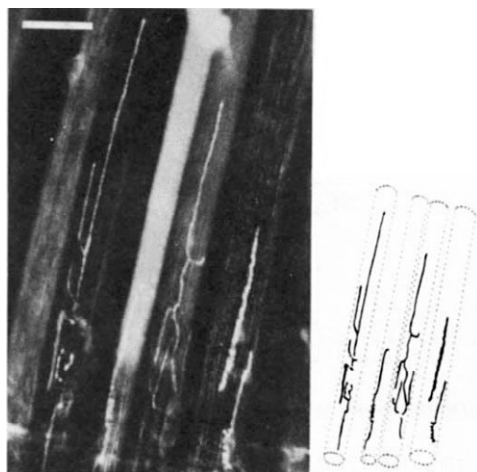


Fig. 1 Fluorescence photomicrograph of endplates of living frog cutaneous pectoris muscle stained with $\text{DiOC}_2(5)$. Note the variety of terminal morphologies and variation of staining patterns among muscle fibres. Inset, sketch from the photomicrograph depicting only presynaptic terminals (postsynaptic muscle fibres are indicated by dotted cylinders). The preparation (from *Rana pipiens*) was stained at 22 °C by exposure to 0.5 μM $\text{DiOC}_2(5)$ (Kodak) in frog Ringer's solution for 10 min, followed by rinsing with normal frog Ringer's. The stain remains in the terminals for >24 h. Fluorescence was photographed with a Leitz Dialux microscope equipped with a Ploemopak 2.4 epiilluminator, HBO 50W Hg light source, and M2 filter block (green excitation, red emission). The photograph was made on Ektachrome ASA 200 daylight film with a Wild $\times 20$ 0.6 n.a. dry objective, Leitz $\times 10$ photoeyepiece, and Wild $\times 0.32$ 35 mm camera adapter; exposure time 5 s. Calibration bar, 50 μm .

fluorescence and ZIO patterns in this respect also indicates that the fluorescent stain was localized in the terminals themselves rather than in closely associated Schwann cells. Other details visible in nerve terminals stained with $\text{DiOC}_2(5)$ but only poorly if at all resolved by DIC optics included short projections and transverse branchlets from the main shafts of frog terminals (see Fig. 1) and, at mouse endplates, vermiform fluorescent elements which appeared to be inside the motor nerve terminals and which may have been whole, or parts of, mitochondria (see Fig. 2b).

Figure 3 shows examples of living muscle preparations from frog (a), mouse (b) and larval *Drosophila*⁹ (c) stained with another dye, rhodamine 123 (Rh123). In general, muscle fibres stained by this dye displayed a diffuse, relatively featureless fluorescence compared with those stained by $\text{DiOC}_2(5)$. This was advantageous in the *Drosophila* preparation where nerve terminals were easily seen after staining with Rh123 (Fig. 3c) but were less readily visible against the fluorescence of muscle fibres after staining with $\text{DiOC}_2(5)$.

In initial experiments with a third dye, 3,3'-dihexyloxacarbocyanine iodide [$\text{DiOC}_6(3)$], closely related to but probably more permeant than $\text{DiOC}_2(5)$ (see refs 10,11), the staining of nerve terminals and muscle fibres from frog was similar to that obtained with $\text{DiOC}_2(5)$. However, $\text{DiOC}_6(3)$ rapidly stained a network-like array of cells overlying the muscle and these obscured the details of interest in nerve and muscle. Therefore, $\text{DiOC}_6(3)$ was not examined further.

Tests for physiological effects of staining alone, and of subsequent fluorescence-exciting illumination, were done on frog muscles and using $\text{DiOC}_2(5)$. Staining itself had no apparent effect on spontaneous miniature endplate potentials (m.e.p.ps) or on endplate potentials (e.p.ps) evoked by stimulation of the motor nerve (Table 1, dye addition experiments). Nerve-evoked muscle contractions monitored qualitatively by visual inspection were similarly unaffected. Illumination of $\text{DiOC}_2(5)$ -stained

Table 1 $\text{DiOC}_2(5)$ staining and low-level illumination have no effect on intracellularly recorded synaptic potentials in frog cutaneous pectoris muscle

	Spontaneous m.e.p.ps Amplitude (mV)	Frequency* (s ⁻¹)	Evoked e.p.ps Amplitude (mV)
Dye addition experiments			
Control	0.46 $\pm$ 0.13	1.26 $\pm$ 0.12 (N = 120)	8.03 $\pm$ 0.34 (N = 20)
Stained	0.45 $\pm$ 0.13	1.01 $\pm$ 0.10 (N = 96)	8.01 $\pm$ 0.23 (N = 20)
Illumination experiments			
Control	0.55 $\pm$ 0.12	2.73 $\pm$ 0.21 (N = 170)	11.54 $\pm$ 0.42 (N = 30)
Illuminated	0.57 $\pm$ 0.11	2.53 $\pm$ 0.20 (N = 153)	11.22 $\pm$ 0.31 (N = 30)

For amplitude measurements, the mean $\pm$ s.d. is given. Dye addition experiments: effects of staining alone without illumination. Spontaneous m.e.p.p. responses were recorded from a single fibre for a period just before (control) and after staining. E.p.p. responses were recorded from a separate preparation bathed in solution containing 4 μM (+)tubocurarine to reduce e.p.ps below the threshold for muscle excitation; e.p.ps, produced by continuous stimulation of the nerve at 0.5 Hz, were recorded from a single fibre just before (control) and after staining. Staining in both cases involved exposure to 0.5 μM $\text{DiOC}_2(5)$ in Ringer's for 5–10 min, followed by rinsing with normal Ringer's. Illumination experiments: effects of low-level fluorescence-exciting illumination. M.e.p.ps and e.p.ps were measured as in the dye addition experiments except that responses were obtained before (control) and during illumination of fibres already stained with $\text{DiOC}_2(5)$; staining as described above. Optics were as described in Fig. 2 legend but with a lower intensity light source (a 12 V, 100 W tungsten-halogen lamp operated at ~ 9 V). The fluorescence of the nerve terminal was clearly visible during illumination.

* N/total time $\pm$ s.d. expected (Poisson) for samples of size N.

endplates at levels sufficient for clear fluorescence visibility of terminals also did not detectably alter m.e.p.ps or e.p.ps, provided that the illumination was kept to a brief duration or low intensity (Table 1, illumination experiments). However, greater exposure (for example more than a few seconds with the optics and Hg source of Fig. 2) of stained, but not of unstained, preparations produced increases in m.e.p.p. frequency that were not reversed by darkness. Increase in m.e.p.p. frequency can result from an elevation in free Ca^{2+} concentration within nerve terminals, and such an elevation could be produced by Ca^{2+} released from illuminated, dye-sensitized mitochondria (see ref. 12). Consistent with this notion is the observation that extracellular Ca^{2+} is not necessary for the effect, that is, increase in m.e.p.p. frequency could be obtained by illuminating stained preparations bathed either in normal Ringer's or in Ringer's containing 2 mM EGTA, 2 mM Mg^{2+} , but no Ca^{2+} .

Continued fluorescence-exciting illumination (for example, several seconds of illumination as in Fig. 2) also produced changes in the fluorescence appearance of stained preparations. Initially the visibility of nerve terminals was enhanced, often markedly, by a selective increase in their fluorescence intensity. Eventually, there was a slow fading of all fluorescence. The enhancement of terminal fluorescence occurred more rapidly with stronger illumination, could be sharply localized (within a few μm) to one part of a terminal by focal illumination, and sometimes clearly revealed terminals that were only barely or not at all visible on first inspection. A dramatic change often seen during illumination of frog muscle fibres stained with $\text{DiOC}_2(5)$ was the induction of a series of fluorescence intensity fluctuations ('twinkle') in the subcellular elements (mitochondria?) within fibres. Individual elements, sometimes initially invisible, would brighten, undergo a series of fluctuations (periodicity on the order of a second), and then disappear. The effect was restricted to long-illuminated regions, and it seemed more easily elicited in areas bordering previously illuminated ones.

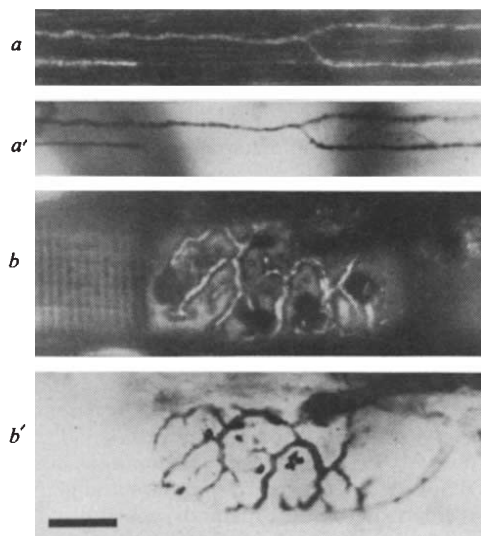


Fig. 2 Fluorescence photomicrographs of endplate areas in living frog cutaneus pectoris (*a*) and mouse triangularis sterni (*b*) muscles stained with DiOC₂(5), and bright-field photomicrographs of the same areas after fixing and staining with ZIO (*a'*, *b'*). Precise alignment of the images was precluded by tissue distortion during the ZIO processing, but the detailed branching pattern of presynaptic terminals revealed by the two stains is virtually identical in each case. There is also excellent correspondence of the striated or beaded appearance of the frog terminal with both stains (*a*, *a'*). The broad, dark vertical bands in *a'* are ZIO-stained nerves out of focus. Note the vermiform elements (mitochondria?) visible within the mouse nerve terminal in the fluorescence photomicrograph (*b*); dark, oval shadows in the same photomicrograph probably represent muscle nuclei devoid of dye. The preterminal mouse axon (entering from the upper right), isolated blobs and the cluster of blobs that are not associated with the terminal were stained by ZIO (*b'*) but not by the fluorescent dye (*b*). DiOC₂(5) staining was as described in Fig. 1 legend with mammalian Ringer's used for the mouse preparation. ZIO staining was as described in ref. 8. Fluorescence and photographic equipment were as for Fig. 1 except that a Zeiss $\times 40$ 0.75 n.a. water-immersion objective was used, and the film was Tri-X (processed for ASA 600); fluorescence exposure 3 s. Calibration bar, 20 μ m for all.

Both the enhancement and twinkling phenomena were observed in preparations that had been well rinsed after staining. Thus, it seems likely that they reflected changes in aspects of the local environment which affect fluorescence (for possible examples see refs 13, 14), rather than changes in local dye quantity.

The fluorescence epiillumination system used in the above *in vitro* experiments also permitted visualization of superficial nerve terminals on muscles in intact, live animals. This was demonstrated with the frog sartorius muscle. The loose thigh skin of an anaesthetized (0.5% tricaine) frog was cut and reflected to expose the sartorius muscle which was stained with 0.5 μ M DiOC₂(5) for 10 min and rinsed with normal Ringer's. Bright-field epiillumination with crossed polarizing filters was then used to trace nerve bundles to areas likely to have superficial endplates. Epifluorescence inspection of such areas revealed staining of terminals and muscle fibres like that obtained with the *in vitro* preparations. In a toxicity test, a frog which received a subcutaneous injection of 1 ml of 10 μ M DiOC₂(5) (far in excess of amounts shown effective for staining by such injection) has survived for months without any apparent ill effects. The technique may thus be useful for chronic studies in intact animals, for example, of synaptic development and turnover.

Our experiments demonstrate that not only do the fluorescent dyes allow living nerve terminals to be viewed with greater clarity than previously possible, but they can do so without apparent compromise to the physiological integrity of the preparation. There are, however, several unresolved issues and limitations in the approach: (1) The explanation for the selective

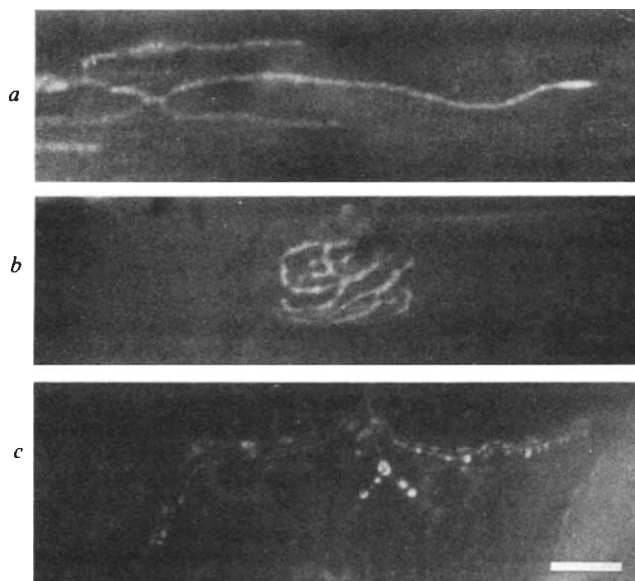


Fig. 3 Fluorescence photomicrographs of neuromuscular junctions in living muscles of frog (*a*), mouse (*b*) and larval *Drosophila* (*c*) stained with Rh123. The longitudinal axis of the underlying muscle fibre is horizontal in each case. Only a portion of the frog terminal is shown. Note that the *Drosophila* nerve terminates in a series of varicosities or boutons, some with dark centres. The preterminal axon is visible for *Drosophila*, entering from the top centre of the photograph. Preparations were stained by exposure for 10 min to 26 μ M Rh123 (Kodak, laser grade) in the appropriate Ringer's followed by thorough rinsing with the same Ringer's. Rh123 fluorescence was observable with either the Leitz M2 (as in Figs 1, 2) or H2 (blue excitation, green emission) filter blocks. Fluorescence and photographic equipment were as described for Fig. 2, except that the mouse preparation was photographed with filter block H2, others with block M2; the exposures of the frog and mouse terminals were similar to those for Figs 1, 2, that of the *Drosophila* terminal was approximately five times longer. Calibration bar, 20 μ m, applies to all.

staining of nerve terminals remains uncertain. The working hypothesis is that it depends on uptake and trapping of the dyes by mitochondria¹⁻³. However, it remains to be determined whether mitochondria are in fact the principal dye-binding elements in terminals, and if they are, which of their properties account for the brightness of terminal fluorescence. (2) The possibility of long-term or subtle biological effects of the dyes, or illumination of them, remains to be explored in these preparations. Various effects of such dyes have been described in other systems (see reviews in refs 13, 14). These studies, however, usually involved higher dye concentrations and illumination intensities than described here, and we have found that terminals can be readily visualized with further reductions of dye and light (to <0.1 , each, of the levels indicated in Table 1) by using a high-sensitivity video camera. (3) With the epiillumination system used here, good visualization was restricted to superficial nerve terminals; these are, however, the ones most accessible to experimental probing with microelectrodes. (4) Even among superficial terminals, we encountered considerable variability in fluorescence visibility. The origin and significance of this variability is unknown, and although it presents a technical inconvenience, it may reflect interesting differences of type or physiological state among terminals.

In addition to their potential application for staining living nerve terminals *in vitro* and *in vivo*, other, related uses of the dyes warrant consideration. It has been suggested² that staining by the dyes might vary with cellular activity, presumably because of the effect of energy stress on mitochondria. If this proves

correct, the dyes could provide useful optical markers for active (or recently active) excitable cells or cell parts. The known influence of cell membrane potential on the entry of these and similar dyes^{10,11,13,14} offers an alternative approach to the same end. In addition, as some nerve terminal functions (for example, transmitter release) can be sensitized to light by the dyes, the labelling may offer opportunities for focal perturbations of selected synaptic activities.

Preliminary experiments suggest that presynaptic boutons on principal neurones in parasympathetic cardiac ganglia in the frog¹⁵ may also be stained by the fluorescent dyes used here. Thus, the utility of the fluorescence approach does not seem to be confined to neuromuscular synapses.

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1. Johnson, L. V., Walsh, M. L. & Chen, L. B. *Proc. natn. Acad. Sci. U.S.A.* **77**, 990–994 (1980).
2. Johnson, L. V., Walsh, M. L., Bockus, B. J. & Chen, L. B. *J. Cell Biol.* **88**, 526–535 (1981).
3. Hetherington, D. C. *Stain Technol.* **11**, 153–154 (1936).
4. Gray, E. G. & Guillery, R. W. *Int. Rev. Cytol.* **19**, 111–182 (1966).
5. Peters, A., Palay, S. L. & Webster, H. de F. *The Fine Structure of the Nervous System: The Neurons and Supporting Cells* (W. B. Saunders, Philadelphia, 1976).
6. McMahan, U. J., Spitzer, N. C. & Peper, K. *Proc. R. Soc. B* **181**, 421–430 (1972).
7. McArdle, J. J. *et al. J. Neurosci. Meth.* **4**, 109–115 (1981).
8. Akert, K. & Sandri, C. *Brain Res.* **7**, 286–295 (1968).
9. Jan, L. Y. & Jan, Y. N. *J. Physiol., Lond.* **262**, 189–214 (1976).
10. Sims, P. J., Waggoner, A. S., Wang, C. H. & Hoffman, J. F. *Biochemistry* **13**, 3315–3330 (1974).
11. Waggoner, A. S., Wang, C. H. & Tolles, R. L. *J. Membrane Biol.* **33**, 109–140 (1977).
12. Alnaes, E. & Rahamimoff, R. *J. Physiol., Lond.* **248**, 285–306 (1975).
13. Waggoner, A. S. *A. Rev. Biophys. Bioengng* **8**, 47–68 (1979).
14. Freedman, J. C. & Laris, P. C. *Int. Rev. Cytol. Suppl.* **12**, 177–246 (1981).
15. McMahan, U. J. & Kuffler, S. W. *Proc. R. Soc. B* **177**, 485–508 (1971).

Gelsolin inhibition of fast axonal transport indicates a requirement for actin microfilaments

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The actions of actin-based microfilaments in cell motility suggest a possible role in the mechanism of fast axonal transport^{1–3}, but the pharmacological data evaluating their role in this process are equivocal^{4–6}. Moreover, microfilaments are difficult to preserve and identify in ultrastructural studies⁷, so the organization and function of axonal actin has remained uncertain. We have now evaluated the role of actin microfilaments in intracellular transport of membranous organelles using video-enhanced contrast microscopy and gelsolin to analyse fast axonal transport directly in isolated axoplasm from the squid giant axon. With this preparation it is possible to perfuse axoplasm with large molecules that do not cross the plasmalemma, while controlling cation levels. The 90,000-molecular weight protein gelsolin depolymerizes actin microfilaments in micromolar Ca^{2+} , but not in the absence of Ca^{2+} . Axonal transport of membranous organelles has previously been shown to be unaffected by levels of Ca^{2+} up to $10\text{ }\mu\text{M}$ ⁸. In the presence of EGTA, gelsolin has no effect on the movement of membranous organelles, but in the presence of $10\text{ }\mu\text{M}$ Ca^{2+} it completely blocks transport of all membranous organelles. No changes in the organization of the axoplasm were detected. These results and results using other probes for actin are consistent with the hypothesis that actin-based microfilaments are involved in the movement of membranous organelles in the axon.

Fast axonal transport (FAT) can be inhibited in neurones by the injection of DNase I^{3–5} and filamin³. DNase I depolymerizes microfilaments by disrupting the F-actin/G-actin equilibrium⁹, whereas filamin inhibits activation of the myosin ATPase¹⁰. However, due to difficulties in controlling concentration and distribution, the injection of materials into neurones cannot rule out the possibility that the role of microfilaments is primarily structural and that inhibition of transport is secondary to disruption of axoplasmic organization^{2,6}. Cytochalasins induce partial depolymerization of microfilaments *in vitro*^{11,12}, but do not inhibit FAT consistently^{4,5}. However, cytochalasins appear to require free microfilament ends to function^{11,12} and these may not be exposed on axonal microfilaments^{13,14}. Phalloidin stabilizes microfilaments and disrupts cellular processes that require disassembly of microfilaments¹⁵, but also fails to inhibit FAT^{6,16}.

Video-enhanced contrast differential interference contrast optics make it possible to detect structures as small as individual microtubules^{17,18}. The full range of membranous organelles can be detected in isolated axoplasm from the squid giant axon^{19,20}, using these video microscopic methods, which provide a means of directly evaluating the effects of agents on both FAT and axoplasmic order in conditions where the microenvironment and concentrations of the active agents can be rigorously controlled. Axoplasm may be perfused with appropriate buffers without inhibiting transport, allowing the use of pharmacological probes that would not cross a plasma membrane^{8,19}. The concentrations and ionic milieus of these agents may be controlled because perfusion is an equilibrium process between known volumes, and the effects of the probes and changes in incubation conditions can then be directly analysed. The entire process of perfusion and incubation is conducted while the axoplasm is under observation.

Gelsolin is present in a wide variety of tissues²¹, and the ability to 'switch' gelsolin activity on and off by small changes in Ca^{2+} concentration makes it a powerful probe for actin microfilaments. As FAT in isolated axoplasm is unaffected by changes in Ca^{2+} levels⁸ from 5 mM EGTA to $10\text{--}100\text{ }\mu\text{M}$ Ca^{2+} , any effect of gelsolin on the movement of membranous organelles in the presence of Ca^{2+} that is not found in the

Table 1 Effect of gelsolin and DNase I on the movement of membranous organelles in isolated axoplasm

Conditions	Movement of particles in FAT after 30 min	Conditions	Movement of particles in FAT after 30 min
Control	+++	$10\text{ }\mu\text{M}$ CaCl_2 in buffer X	+++
1 mM EGTA in buffer X	+++	Gelsolin + $10\text{ }\mu\text{M}$ CaCl_2 in buffer X	0
Gelsolin + 1 mM EGTA in buffer X	+++	10 mg ml^{-1} DNase I in buffer X	--

Isolated axoplasm from squid giant axons was prepared as described previously^{8,19} sandwiched between two coverslips with proximal and distal ends left unsealed to permit perfusion. Preparations were examined for FAT activity and FAT recorded for 10 min or more before perfusion to serve as a basis for comparison. $20\text{--}25\text{ }\mu\text{l}$ of buffer X (350 mM potassium aspartate, 130 mM taurine, 70 mM betaine, 50 mM glycine, 12.9 mM MgCl_2 , 20 mM HEPES, 5 mM potassium EGTA, 1 mM glucose, 1 mM potassium ATP, pH adjusted to 7.2 with KOH) modified as described below and in the text was perfused into the proximal end and $5\text{ }\mu\text{l}$ drawn from the distal end to ensure filling of the incubation chamber. Video records of 5-min segments were made at 10, 20 and 30 min to document changes in FAT. Only effects noted within 30 min were scored. Video records were then evaluated for the amount of material moving in FAT and overall changes in the appearance of the axoplasm. +++, Not distinguishable from control experiments in similar perfusion conditions in either amount or velocity of particle movement; 0, no movements identifiable as FAT; --, severe disruption of both FAT and organization of the axoplasm.

absence of Ca^{2+} should be due to the effect of gelsolin on axoplasmic microfilaments.

Rabbit macrophage gelsolin was purified (Fig. 1) by methods described previously^{21,22}. For perfusion of the axoplasm, gelsolin was diluted in either EGTA buffer X (see Table 1 legend for composition) or 10 μM Ca^{2+} buffer X (buffer X except with

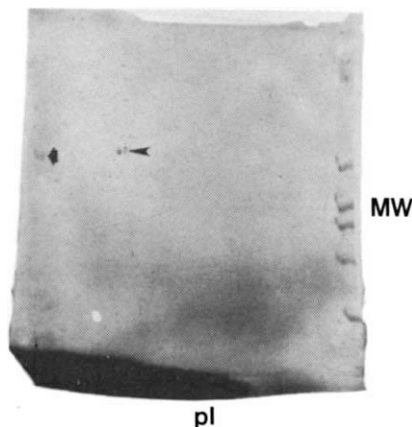


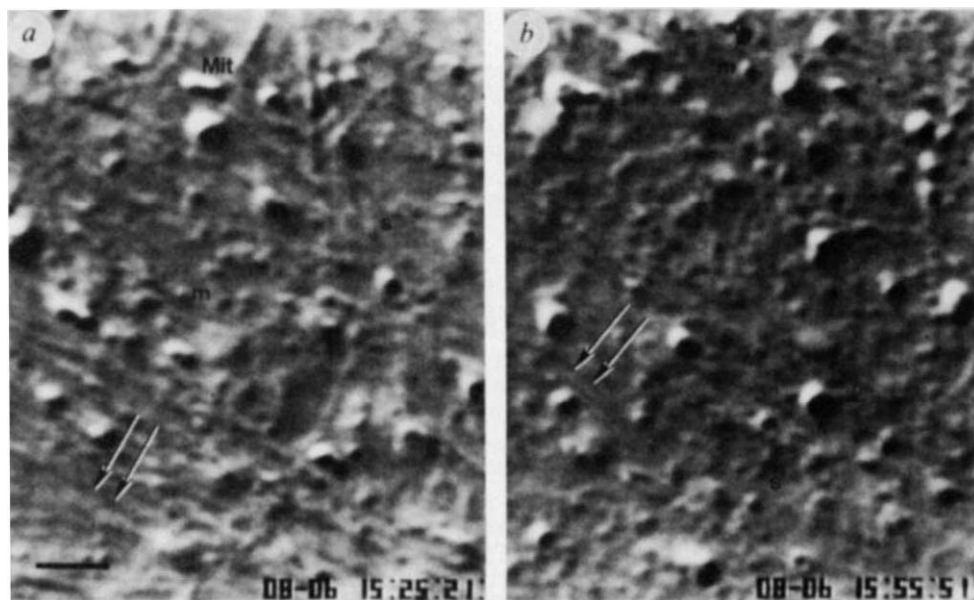
Fig. 1 One- and two-dimensional gel electrophoresis of the gelsolin samples used in these studies. Samples of the purified rabbit macrophage gelsolin were analysed in both discontinuous SDS-polyacrylamide gel electrophoresis (PAGE) modified slightly from the method of Laemmli²⁴ and two-dimensional PAGE as modified from O'Farrell²⁵, to demonstrate purity and to estimate concentrations of gelsolin used. Activity against microfilaments *in vitro* was comparable with that reported previously^{21,23}. The figure shows the Serva brilliant blue-stained pattern of the gelsolin used in these studies analysed in both SDS-PAGE (large arrow) and two-dimensional PAGE (arrowhead). The right-hand side of the gel contains molecular weight standards of 14,000, 30,000, 43,000, 57,000, 68,000, 92,000 and 200,000, respectively. Ampholytes (Pharmacia) covering the pH range of 5–7 were used and acidic proteins are towards the right. In this gel system, gelsolin runs at a molecular weight of 90–92,000 with an isoelectric doublet in the pH range 6.1–6.3 (see also ref. 22). Approximately 0.1–0.2 μg of gelsolin was perfused into the axoplasm in a typical experiment at a concentration of 0.01–0.02 mg ml^{-1} . Actin is present in axoplasm at a concentration of 1–1.5 mg ml^{-1} (ref. 13). Perfusion chambers were generally arranged so that the incubation volumes of buffer were approximately equal to the volume of axoplasm in a given preparation. The ratio of actin to gelsolin approaches 100:1 in these experiments.

10 μM CaCl_2 and no EGTA). Buffer X is modelled after the small molecular weight composition of squid axoplasm^{13,14}. Organization of the axoplasm is maintained and FAT continues at control levels in buffer X. Gelsolin was equilibrated for 30–60 min in the appropriate buffer before perfusion to ensure that it was in the desired state of activation.

Isolated axoplasm was prepared as described previously for video-enhanced contrast differential interference contrast microscopy^{8,19}, a suitable region chosen for observation, and FAT recorded for 5–10 min to serve as a baseline for comparison. The isolated axoplasm was perfused with 10 μl of the appropriate gelsolin–buffer X mixture (approximately twice the volume of the axoplasm), and preparations were observed continuously for at least 30 min after perfusion. Equilibration of a protein the size of gelsolin would be expected to be complete within 10–20 min of perfusion¹³. No effects on FAT or organization of the axoplasm were noted with gelsolin and EGTA–buffer X (Table 1). When gelsolin and 10 μM Ca^{2+} buffer X were perfused, however, both numbers and velocities of transported particles were noticeably inhibited after 20 min. Inhibition of particle movement occurred rapidly once begun and was generally complete within 5 min of first detectable inhibition. By 30 min, there was an effectively complete inhibition of transport for all sizes of particles. The organization of the axoplasm remained essentially normal (Table 1, Fig. 2a, b). In all gelsolin experiments, FAT was blocked within 30 min in the presence of micromolar Ca^{2+} and was unaffected in the presence of EGTA. Consistent with observations obtained by injection^{3–6}, DNase I (Schwarz-Mann) at a concentration of 5–7 mg ml^{-1} in the axoplasm (considerably in excess of axoplasmic concentrations of actin, 1.0–1.5 mg ml^{-1} (ref. 13)) inhibited FAT, but induced a concomitant disruption of the linear organization of the axoplasm.

The only documented activity of gelsolin is the break-up of actin microfilaments, which is activated by micromolar concentrations of Ca^{2+} . Other activities may be described in the future, but the specificity of action for gelsolin, the low levels of gelsolin required for an effect, and the correlation of Ca^{2+} dependence between microfilament depolymerization and inhibition of FAT all argue that the effect of gelsolin on FAT results from an action on axoplasmic microfilaments. The action of DNase I is also consistent with this hypothesis. All sizes of membranous organelles, including 50-nm vesicles, mitochondria and organelles moving both in orthograde and retrograde direc-

Fig. 2 Effects of gelsolin and 10 μM Ca^{2+} buffer X on the organization of the axoplasm. Stills from the video record indicate that the overall appearance of axoplasm just after perfusion (a) is virtually indistinguishable from that after 30 min incubation with gelsolin in the presence of 10 μM Ca^{2+} (b). Examples of the different classes of membranous organelles are indicated, including mitochondria (Mit), medium-sized particles that move primarily in the retrograde direction (m) and small particles that move primarily orthograde (s). Linear elements are indicated by the double arrows. No changes were detected in the distribution of either membranous organelles or the linear elements that correspond to microtubules, neurofilaments and associated materials. At the time the photograph in b was taken, there was effectively no movement of membranous organelles in FAT (see Table 1) and minimal evidence of brownian movements typically seen when the organization of the axoplasm is disrupted and membranous particles are freely moving in an aqueous medium. Prolonged incubations with gelsolin and Ca^{2+} or treatment with high concentrations of DNase I for less than 30 min results in a disorganization of the linear elements and a significant increase in the amount of brownian movements. No translocation of organelles is seen that corresponds to FAT in the control axoplasm after treatment with high concentrations of DNase I.



tions, were affected equally, indicating a common microfilament-associated mechanism for movement. The preservation of order after inhibition of FAT by gelsolin argues against an indirect inhibition produced by disruption of axoplasmic order, although disruption of axoplasm by high concentrations of DNase I suggests that microfilaments may also be involved in maintaining the organization of axoplasm. The relative preservation of order with gelsolin may be because it does not produce a complete loss of microfilaments, but only shortening of existing microfilaments²³.

Ca²⁺-dependent inhibition of FAT by gelsolin indicates that actin is involved in the movement of membranous organelles in

axonal transport, but more details about the role of related polypeptides, such as myosin, are required before a complete model for the molecular mechanisms of FAT can be proposed. Future experiments with the isolated axoplasm preparation will be directed towards an understanding of the role of other cytoskeletal proteins in the organization of the axoplasm and the movement of membranous organelles in axonal transport.

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1. Bray, D. *Biochimie* **59**, 1–6 (1977).
2. Goldberg, D. in *Axoplasmic Transport* (ed. Weiss, D.) 73–80 (Springer, Berlin, 1982).
3. Isenberg, G., Schubert, P. & Kreutzberg, G. in *Axoplasmic Transport* (ed. Weiss, D.) 314–321 (Springer, Berlin, 1982).
4. Isenberg, G., Schubert, P. & Kreutzberg, G. *Brain Res.* **194**, 588–593 (1980).
5. Goldberg, D., Harris, D., Lubit, B. & Schwartz, J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7448–7452 (1980).
6. Goldberg, D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4818–4822 (1982).
7. Forer, A. *Meth. Cell Biol.* **25**, 131–142 (1982).
8. Brady, S., Lasek, R. & Allen, R. *Cell Motility* (submitted).
9. Lazarides, E. & Lindberg, U. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4742–4746 (1974).
10. Davies, P., Bechtel, P. & Pastan, I. *FEBS Lett.* **77**, 228–232 (1977).
11. Brown, S. & Spudis, J. *J. Cell Biol.* **88**, 487–491 (1981).
12. McLean-Fletcher, S. & Pollard, T. *Cell* **20**, 329–341 (1980).
13. Morris, J. thesis, Case Western Reserve Univ. (1981).
14. Morris, J. & Lasek, R. *J. Cell Biol.* **92**, 192–198 (1982).
15. Wehland, J., Osborn, M. & Weber, K. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5613–5617 (1977).
16. Brady, S. T., Morris, J. R. & Lasek, R. J. (in preparation).
17. Allen, R., Allen, N. & Travis, J. *Cell Motility* **1**, 291–302 (1981).
18. Allen, R. D. & Allen, N. J. *Microsc.* **129**, Pt 1, 3–17 (1983).
19. Brady, S., Lasek, R. & Allen, R. *Science* **218**, 1129–1131 (1982).
20. Allen, R., Metzuzals, J., Tasaki, I., Brady, S. & Gilbert, S. *Science* **218**, 1127–1129 (1982).
21. Yin, H., Albrect, J. & Fattoum, A. *J. Cell Biol.* **91**, 901–906 (1981).
22. Yin, H. L., Kwiatkowski, D., Mole, J. & Cole, F. J. *J. Biol. Chem.* **259**, 5271–5276 (1984).
23. Yin, H., Hartwig, J., Maruyama, K. & Stossel, T. J. *J. Biol. Chem.* **256**, 9693–9697 (1981).
24. Laemmli, U. *Nature* **227**, 680–685 (1970).
25. O'Farrell, P. J. *J. Biol. Chem.* **250**, 4007–4021 (1975).

Persistent, directional motility of cells and cytoplasmic fragments in the absence of microtubules

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Directional cell locomotion is displayed by many cell types both *in vivo* and *in vitro*¹. In many instances, persistency and directionality are imposed by external stimuli such as chemical attractants or substrate properties^{2–6}. Some cell types, such as fibroblasts or leukocytes, are capable of migrating in the absence of known stimuli in a pattern known as persistent random walk⁷, where the direction of movement is maintained for at least one cell diameter before the cell performs a sudden directional change. In many examples of persistent motility, microtubules are believed to have a key role as elements that stabilize or even determine a cell's direction of movement^{8–11}. If disassembled, persistency is reduced or impaired^{12–15}. Despite some reports to the contrary^{16–18}, these and other observations have led to the widely accepted view that microtubules may be the overall organizers of cell geometry, polarity and motile activity¹⁹. Here we report that rapid, directional locomotion of fish epidermal keratocytes is independent of the presence of microtubules. Moreover, small cytoplasmic fragments derived from the anterior lamella of these cells are capable of locomoting in a pattern indistinguishable from that of intact cells. Since these fragments contain no nucleus, microtubules or centrioles, the persistency-determining component must be sought in some other component(s) of the cytoplasm, possibly the motile machinery of the lamella itself.

Explants of epidermal tissue from goldfish (*Carassius*), *Midas* cichlid and angelfish (*Pterophyllum*) were obtained by collagenase digestion of isolated scales and allowed to attach to glass coverslips in the presence of fish Ringer's solution supplemented with 20% amphibian culture medium. Within 5–20 min, filament-containing epidermal cells (henceforth called keratocytes; see ref. 20) began to emigrate from the explant. These cells were the predominant cell type in fish epidermis²¹; they stained brightly with antibodies against prekeratin in immunofluorescence microscopy and showed prominent bundles of 8–10-nm filaments by electron microscopy, and were thus easily identified. Migration of these cells was characterized by various unusual features. Locomoting cells extend a broad, exceedingly flat and virtually featureless lamellipodium at the

cell anterior, measuring approximately 15×40 μm and 0.1–0.3 μm thick. All major cell organelles, including the nucleus and the bulk of the membrane-bounded organelles, are contained in the large, bulbous cell body located at the cell posterior (Fig. 1). Thus, locomoting cells have a characteristic canoe shape²² also known from amphibian epidermal cells^{23–25}, and they move forward in the direction of their smallest diameter. Only locomoting cells have this appearance; stationary cells are rounded (pancake-shaped) or multipolar. These cells move exceedingly rapidly, averaging 0.5 μm s⁻¹ and occasionally reaching over 1 μm s⁻¹. They are thus among the fastest-moving eukaryotic cells known.

As demonstrated by immunofluorescence and electron microscopy, neither keratin-type intermediate filaments nor microtubules extend into the lamella in moving cells, but instead are contained in the cell body, along with the centrosome. Microtubules are wrapped around the nucleus (Fig. 1). Within the lamella, actin filaments form a delicate criss-cross pattern as seen by labelling with rhodamine-phalloidin (Fig. 1). In some cells small phalloidin-labelled fibres that correspond to small filament bundles extend parallel to the long axis of the cell within the cell body. Extensive filament cables or stress fibres and a 'tail' characteristic of fibroblastic cells are absent, as are ruffles, microspikes or filopodia. Thus, the contractile machinery of epidermal keratocytes is much less complex than that of, for example, fibroblasts, possibly being reduced to the most essential components.

Table 1 Persistency and speed of locomotion of intact keratocytes and lamellar fragments in the presence and absence of microtubule inhibitors

	Persistency factor	
	Untreated cells	Treated cells
Intact cells	1.13 ± 0.10 (n = 10)	1.11 ± 0.11 (n = 11)
Fragments	1.18 ± 0.12 (n = 15)	1.16 ± 0.08 (n = 7)
	Speed (μm min ⁻¹)	
	Untreated cells	Treated cells
Intact cells	35.1 ± 13 (range 20–63; n = 16)	34.2 ± 8 (range 21–50; n = 12)*
Fragments	22.4 ± 9 (range 17–42; n = 14)	23.3 ± 7 (range 18–39; n = 7)†

The persistency factor is defined as: distance travelled/straight-line distance between the starting point and the end point of interval. This factor is a measure of the 'waviness' of cell movement. Drug treatment was for 45 min at 0 °C with 4 μg ml⁻¹ nocodazole or colcemid, followed by 30 min at room temperature in the presence of the drug.

* P > 80%; † P > 70%.

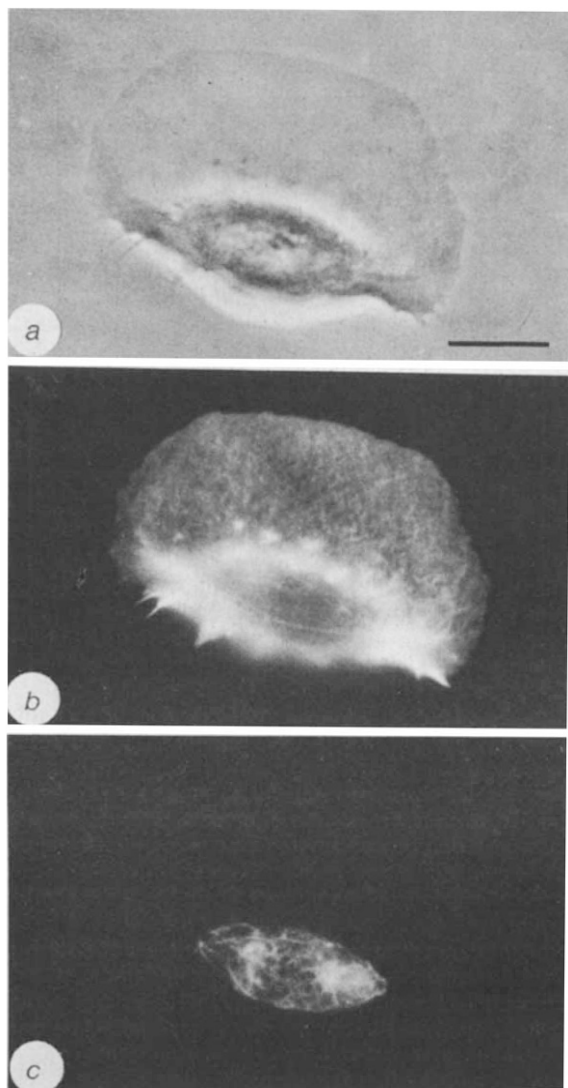


Fig. 1 Phase contrast (*a*) and fluorescence micrographs (*b*, *c*) of a locomoting *Midas* keratocyte in culture. The cell was fixed for 10 min with 1% glutaraldehyde in PHEM buffer³² supplemented with 0.1% Triton X-100, treated with borohydrate³³ for 5 min, and processed for double-label immunofluorescence microscopy with rhodamine-phalloidin³⁴ and an antibody against tubulin. Experiments in which cells were fixed first and then permeabilized with detergent yielded essentially the same results. Note the delicate criss-cross actin weave in the lamella (*b*) and the total absence of microtubules from the lamellar cytoplasm (*c*). Instead, microtubules are wrapped around the nuclear area as can be seen by focusing through the bulbous cell body. Scale bar, 10 μ m.

In their attempt to break loose from a fresh explant, keratocytes at the periphery of a cell cluster extend lamellae over the free substratum. These lamellae may elongate and finally detach or pinch off, while the cell body is still held within the cluster; thus, a motile lamellar fragment is formed. Alternatively, fragments may be generated by cutting off lamellae with fine glass microcapillaries. Many of these lamellar fragments, which constitute between 4 and 10% of the total cell volume, continue to move in a pattern and with a degree of persistency indistinguishable from intact cells. A simple criterion to determine persistency is to compute a 'persistency factor' by dividing the total path length travelled by the net distance achieved during a 10-min time interval (corresponding to about 200–300 μ m or at least 15 cell diameters). The persistency factor of both intact cells and fragments does not exceed 1.20 (Table 1), indicating that there is <20% deviation from the shortest possible route (a straight line between the beginning and the end

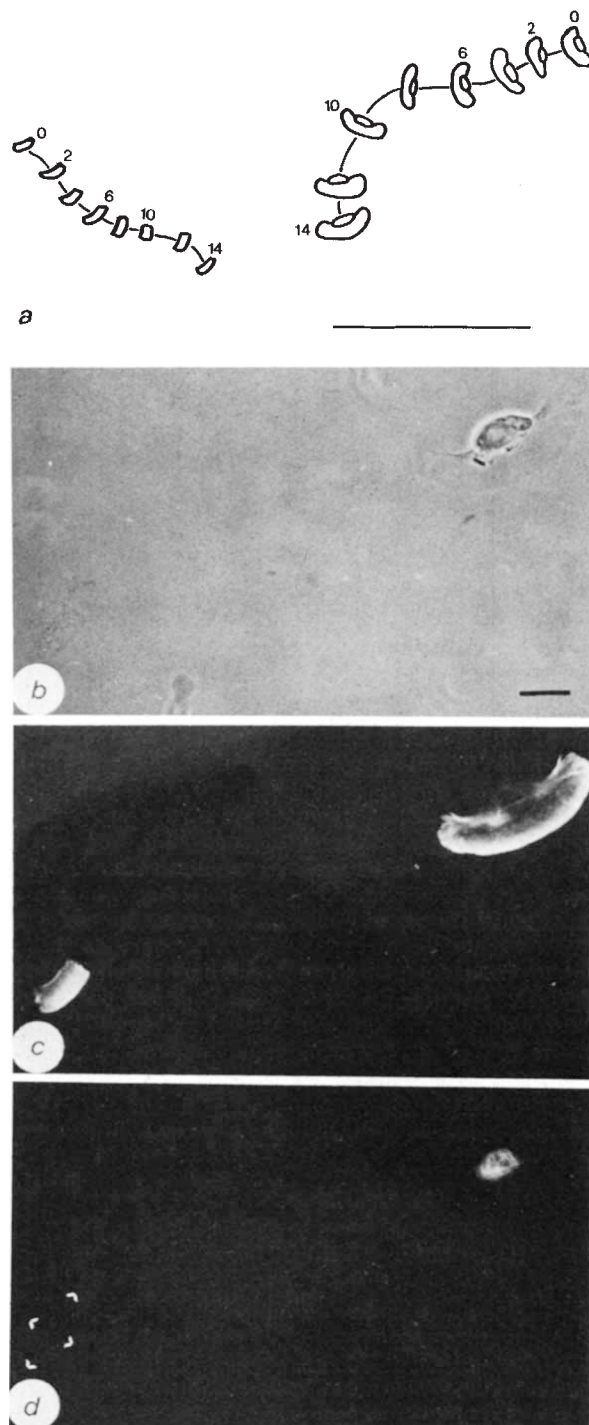


Fig. 2 The movement of one lamellar fragment and one intact cell was followed in a Zeiss IM 35 inverted microscope using a $\times 25$ objective over 14 min; 35-mm phase contrast micrographs were taken every minute. After 14 min, the cells were fixed and processed for double-label fluorescence microscopy. *a*, Pathway of the fragment (left) and the intact cell (right). Numbers are minutes; scale bar, 100 μ m. *b*, Phase contrast; *c*, rhodamine-phalloidin; and *d*, anti-tubulin fluorescence micrographs of the fragment and the intact cell. Note the absence of microtubules from the fragment, while the intact cell displays the characteristic circumnuclear microtubule array.

point of movement). The differences in the persistency factors are not statistically significant ($P > 40\%$). Further, intersegmental angle measurements show that the pathways of cells or fragments after 8 min do not deviate more than about 20° from that chosen during the initial 2-min period. Thus, both cells and fragments are far more persistent in their locomotion than are

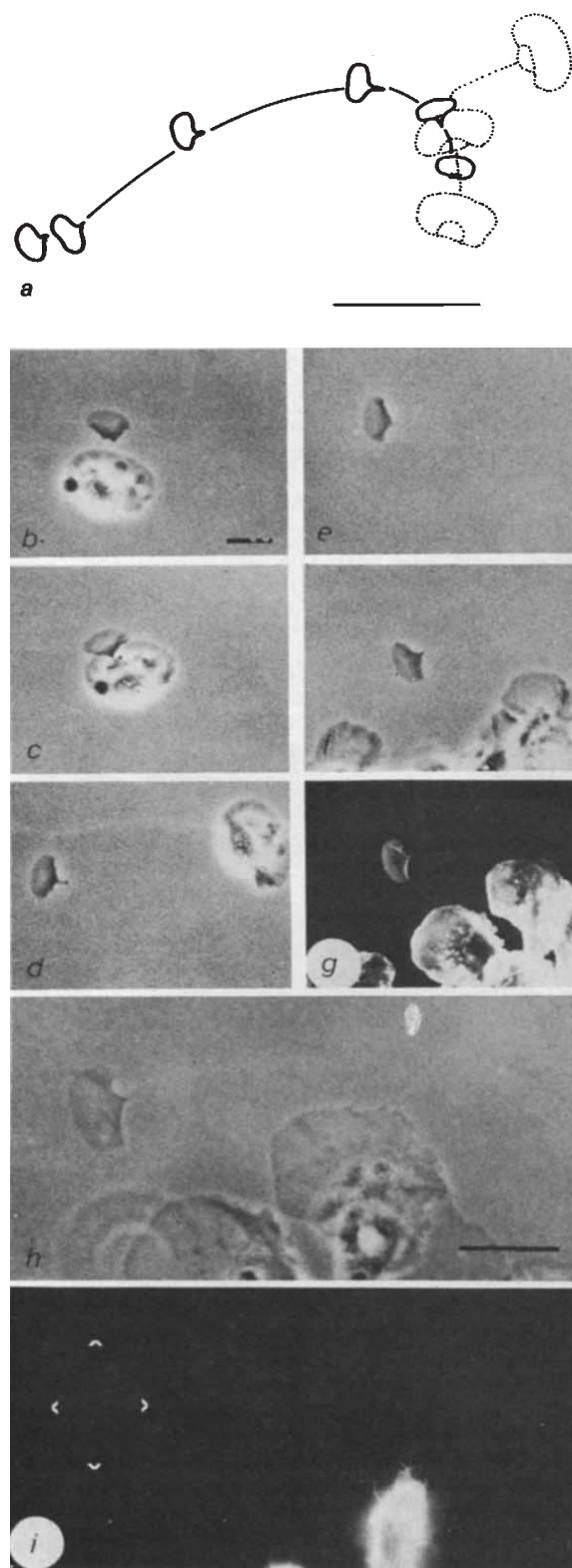


Fig. 3 Tracking the locomotion of a small fragment (a) and corresponding phase contrast micrographs of the living fragment (b-f). An intact cell approaches the fragment from behind (b) and collides with it (c), as a consequence of which both the fragment and the cell change their course (d). The fragment was tracked for another 10 min and then fixed (g). The rhodamine-phalloidin label of the fixed fragment is shown in g at the same magnification as the phase contrast micrographs (b-f). The phase contrast micrograph of the fixed fragment (h) and the anti-tubulin staining (i) are shown at a higher magnification. No microtubules are present. Scale bar in a, 50 μm .

cultured fibroblasts, which frequently and abruptly change direction within 1-2 cell diameters⁷. On the average, fragments move slightly more slowly than intact cells (Table 1).

We have followed the movement of several fragments for a time period of at least 10 min (corresponding to a distance travelled of $\sim 200 \mu\text{m}$) and have then processed these fragments for immunofluorescence microscopy with rhodamine-phalloidin and anti-tubulin to determine the presence and organization of actin filaments and microtubules. Figures 2 and 3 give two examples. The first (Fig. 2) shows the course of a small fragment ($14 \times 18 \mu\text{m}$) recorded over 14 min and then fixed. This fragment, like 7 out of 11 studied in this way, did not contain a single piece of microtubule, while neighbouring intact cells display the characteristic circumnuclear microtubule whorls. The second (Fig. 3) is engaged in a collision with an intact cell, which it avoids successfully by changing its pathway. It, too, contains no microtubules. Four of the fragments did contain one to five short microtubule segments a few micrometres in length; one example is shown in Fig. 4, demonstrating that microtubule fragments are easily identified if they are there. The absence of microtubules was confirmed in six other fragments processed for either serial section electron microscopy or wholemount stereo high-voltage electron microscopy (data not shown). None of a total of over 20 fragments studied by either immunofluorescence microscopy using anti-tubulin or anti-centrosome antibodies or electron microscopy, contained centrioles.

Microtubule pieces within lamellar fragments are probably adventitiously entrapped in the process of fragment formation and are not required for persistent movement. Depolymerization of microtubules by cold treatment in the presence of nocodazole or colcemid (followed by rewarming in the presence of the drugs) does not change persistency or velocity of fragment locomotion in any fragment studied; the same is true for nocodazole- or colcemid-treated intact keratocytes (Table 1). We have followed the movement of several cells treated in this

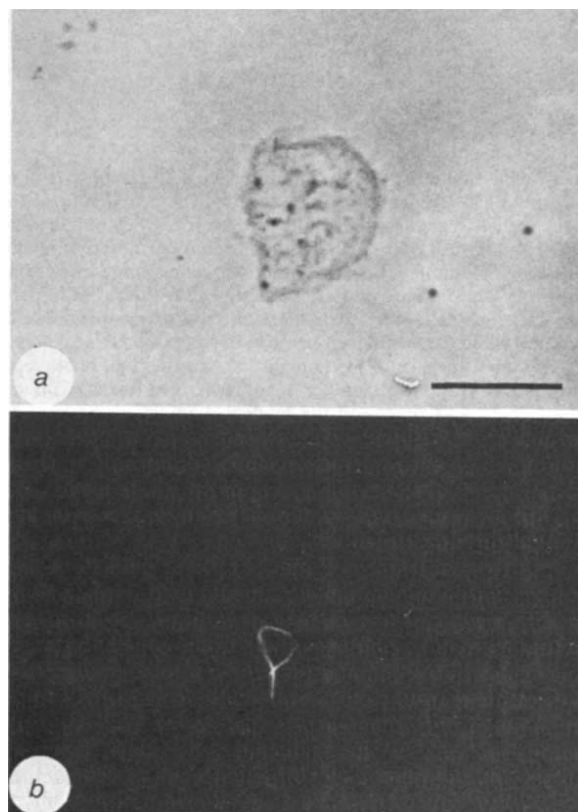


Fig. 4 Phase contrast (a) and anti-tubulin fluorescence micrograph (b) of a fragment that was locomoting before fixation. This fragment contains a single microtubule forming a loop.

way and have processed them for immunofluorescence microscopy. Inhibitor-treated intact cells as well as fragments, which do not possess microtubules, have precisely the same morphology and overall appearance as their untreated counterparts and exhibit the same behavioural repertoires.

This study provides evidence for persistent, directional migration in the absence of microtubules of both intact epidermal keratocytes and cytoplasmic fragments derived from the anterior lamella. Thus, microtubules are not required to choose and maintain the direction of movement, nor are they essential to organize the cytoplasm for motility. Cell fragments of various size that display some form of motile or contractile activity have been experimentally induced previously in a variety of cell types²⁶⁻³¹. Of these, the 'microplasts' of human skin fibroblasts generated by Albrecht-Buehler³⁰ are among the smallest, equal in size to those described here for epidermal cell lamellae. Fibroblast fragments repeatedly display the same type of stereotypic behaviour, such as ruffling or blebbing, without translocating. If these microplasts are the smallest units of general motile activity that cytoplasm is able to express, as suggested by Albrecht-Buehler³⁰, then epidermal lamellar fragments are the smallest units of cytoplasm capable of persistent translocative behaviour. They, too, go through repeated cycles of motile activity, but in this case the activities are spatially and temporally coordinated in such a way that persistent, smooth crawling results. Several conclusions can be drawn from this observation. The force for movement of epidermal cells is generated and regulated locally, in the lamella. The cells possess an autonomous 'front wheel drive' that steers the cell without support or input from framework components of the cell body. The events leading to locomotion are, in principle, independent of external stimuli so that, when left alone, cells and fragments move with a high degree of persistency; they seem to have a 'molecular autopilot'. On the other hand, their pathways can be influenced from outside, as evidenced by the behaviour of contact inhibition after collision.

Our observations draw attention to epidermal lamellae as excellent model systems in which basic mechanisms of cell extension can be studied. Lamellae may represent the essential motile machinery which, in many cell types, is obscured and complicated by epiphenomena such as ruffling, stress fibres, adhesion plaques, cytoplasmic flow or filopodia; in epidermal cells this machinery seems to be expressed in its purest form.

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1. Trinkaus, J. P. in *The Cell Surface in Animal Embryogenesis and Development* (eds Poste, G. & Nicholson, G. L.) 225-329 (Elsevier, Amsterdam, 1976).
2. Zsigmond, S. H. *J. Cell Biol.* **77**, 269-287 (1978).
3. Carter, S. B. *Nature* **208**, 1183-1190 (1965).
4. Dunn, G. A. & Heath, J. P. *Expl Cell Res.* **101**, 1-14 (1976).
5. Gerisch G. et al. in *Development and Differentiation in the Cellular Slime Molds* (eds Cappuccinelli, R. & Ashworth, J. M.) 105-124 (Elsevier, Amsterdam, 1977).
6. Bray, D. in *Cell Behaviour* (eds Bellairs, R., Curtis, A. & Dunn, G.) 299-317 (Cambridge University Press, 1982).
7. Gail, M. H. & Boone, C. *Biophys. J.* **10**, 980-993 (1970).
8. Malech, H. L., Root, R. K. & Gallin, J. I. *J. Cell Biol.* **75**, 666-693 (1977).
9. Albrecht-Buehler, G. *Cell* **12**, 333-342 (1977).
10. Albrecht-Buehler, G. *Cell Motility* **1**, 237-245 (1981).
11. Gotlieb, A. I., McBurnie May, L., Subrahmanyam, L. & Kalnins, V. I. *J. Cell Biol.* **91**, 589-594 (1981).
12. Goldman, R. D. *J. Cell Biol.* **51**, 752-767 (1971).
13. Vasiliev, J. M. & Gelfand, I. M. in *Cell Motility* (eds Goldman, R. D., Pollard, T. D. & Rosenbaum, J. L.) 279-304 (Cold Spring Harbor Laboratory, New York, 1976).
14. Zsigmond, S. H. *J. Cell Biol.* **75**, 606-616 (1977).
15. Gotlieb, A. I., Subrahmanyam, L. & Kalnins, V. I. *J. Cell Biol.* **96**, 1266-1272 (1983).
16. Dipasquale, A. *Expl Cell Res.* **95**, 425-439 (1975).
17. Dunlap, M. K. & Donaldson, D. J. *Expl Cell Res.* **116**, 15-19 (1978).
18. Rich, A. M. & Hoffstein, S. T. *J. Cell Sci.* **48**, 181-191 (1981).
19. Alberts, B. et al. *Molecular Biology of the Cell*, 600-601 (Garland, New York, 1983).
20. Henrikson, R. C. & Matoltsy, A. G. *J. Ultrastruct. Res.* **21**, 194-205 (1968).
21. Schliwa, M. *J. Ultrastruct. Res.* **52**, 377-386 (1975).
22. Goodrich, H. B. *Biol. Bull.* **46**, 252-262 (1924).
23. Radice, G. P. *J. Cell Sci.* **44**, 201-223 (1980).
24. Bereiter-Hahn, J., Strohmeyer, R., Kunzenbacher, I., Beck, K. & Voth, M. *J. Cell Sci.* **52**, 289-311 (1981).

25. Kunzenbacher, I., Bereiter-Hahn, J., Osborn, M. & Weber, K. *Cell Tissue Res.* **222**, 445-457 (1982).
26. Keller, H. U. & Bessis, M. *Nature* **258**, 723-724 (1975).
27. Goldstein, L., Cailleau, R. & Crocker, T. T. *Expl Cell Res.* **19**, 332-342 (1960).
28. Kalisz, B. & Korohoda, W. *Acta protozool.* **15**, 345-357 (1976).
29. Shaw, G. & Bray, D. *Expl Cell Res.* **104**, 55-62 (1977).
30. Albrecht-Buehler, G. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6639-6643 (1980).
31. Swanson, J. A. & Taylor, D. L. *Cell* **28**, 225-232 (1982).
32. Schliwa, M. & van Blerkom, J. *J. Cell Biol.* **90**, 222-235 (1981).
33. Weber, K., Rathke, P. & Osborn, M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1820-1824 (1978).
34. Wulf, E., Deboen, A., Bautz, F. A., Faustich, H. & Wieland, T. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4498-4502 (1979).

Immunoreactive arginine-vasopressin in Brattleboro rat ovary

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Homozygous (*di/di*) Brattleboro rats have normal hypothalamic levels of oxytocin and neurophysin I, but undetectable levels of neurophysin II and arginine-vasopressin (AVP)¹. This defect has been presumed to be at the genomic or transcriptional level, as AVP messenger is reported to be drastically reduced, if not absent, from the hypothalamus of Brattleboro rats². Recent studies suggest *de novo* production of various neuropeptides in the mammalian gonad³⁻⁷, including AVP⁷. We report here the detection and localization of immunoreactive (ir) AVP in the luteal cells of adult homozygous Brattleboro rats, and the modulation of this ovarian ir-AVP by gonadotropins. These findings are thus consistent with a tissue-specific defect of AVP expression in the magnocellular neurones of the Brattleboro rat, and suggest that a comparable defect does not occur in the ovaries of such animals.

Hypothalamic (HT), neurointermediate lobes (N-IL) and ovaries from adult homozygous (*di/di*) Brattleboro rats (200-220 g) and Long-Evans (*LE/LE*) rats (200-230 g) were extracted as described in Fig. 1 legend. The content of AVP-like immunoreactivity was determined by radioimmunoassay using a rabbit antiserum recognizing part or all of the hexapeptide of synthetic AVP (4-9) (antiserum R151)^{8,9}; sensitivity of the AVP assay was routinely 0.5 pg per tube. Serial dilutions of the ovarian extracts from adult *di/di* and *LE/LE* rats showed good parallelism with standard curves of synthetic peptides in the radioimmunoassay system (data not shown); parallel displacement was similarly obtained at higher dilution for extracts of hypothalamus (HT; 1:100) and neurointermediate lobe (N-IL; 1:1,000) prepared from *LE/LE* rats.

The tissue extracts were initially characterized by Sephadex G-50 chromatography (Fig. 1). The gel profiles of ovarian ir-AVP from both adult *di/di* and *LE/LE* rats showed a single distinct peak eluting in the same position as synthetic AVP (1-9); similarly, HT and N-IL extracts of *LE/LE* rats revealed an identical profile of immunoreactivity on chromatography. No AVP-like immunoreactivity was detected in any of the eluate fractions when HT and N-IL extracts from *di/di* rats were chromatographed in identical conditions. In addition, ovarian extracts from adult *di/di* rats were subjected to reverse-phase HPLC (Fig. 2). A broad band of AVP-like immunoreactivity was found, quite distinct from the bulk of the protein, as determined by absorbance at 215 nm; the clear peak of ir-AVP, however, co-eluted with authentic, synthetic AVP. Standard oxytocin (Cambridge Research Biochemicals) eluted as a distinct peak, three fractions later and coincident with the first very minor peak in the vasopressin standard after the major peak.

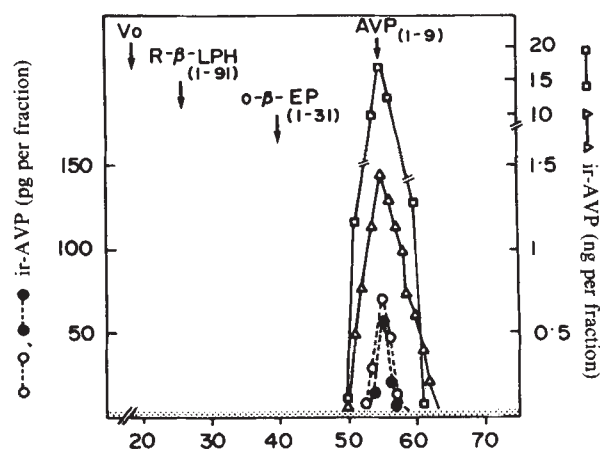


Fig. 1 Chromatographic profiles of immunoreactive vasopressin (ir-AVP), from hypothalamus (Δ), neurointermediate lobe ($\square$) and ovarian extracts ($\circ$) of Long-Evans rats and ovarian extracts ($\bullet$) of homozygous Brattleboro (*di/di*) rats. Hypothalamus ($\times 1$), pituitary ($\times 1$) and pooled ovaries ($\times 12$) were collected in 5 ml of 0.1 M HCl at 4°C. Extracts were then prepared by boiling for 15 min, cooling on ice and homogenizing with a Polytron (Brinkmann Instruments, speed setting 3, 2 $\times$ 3-s bursts). Homogenates were centrifuged (10,000g, Sorvall RC-5, DuPont) for 20 min at 4°C. The supernatant was removed, lyophilized and reconstituted in 5 ml of 1% formic acid, and a 3-ml sample applied to a Sephadex G-50 fine column (1.9 $\times$ 54 cm), equilibrated and eluted at room temperature with 1% formic acid containing bovine serum albumin (0.2 mg ml⁻¹). Fractions (2 ml) were collected, lyophilized, reconstituted in 1 ml assay buffer and assayed with AVP antiserum (R151). The column was calibrated with blue dextran (void volume, V_0), purified human β -lipotropin (β -LPH, gift of Dr P. Lowry), synthetic human β -endorphin (1-31) (β -EP, Peninsula) and synthetic arginine vasopressin (AVP (1-9); Ferring). For radioimmunoassay, ¹²⁵I-vasopressin tracer was prepared by iodination of synthetic AVP (1-9) by a modified chloramine-T method. ¹²⁵I-AVP was collected from an Amberlite IRA-400 anion exchange column, followed by Sephadex G-25 chromatographic purification. Rabbit antiserum raised against synthetic AVP (1-9) is specific for the hexapeptide AVP (4-9), and thus recognizes on an equimolar basis 1-deamino-8-[D-arginine]vasopressin and 1-deamino-8-[D-arginine]vasotocin, but cross-reacts <10% with lysine-vasopressin; it does not recognize oxytocin, angiotensin I, angiotensin II, Met-enkephalin or Leu-enkephalin. Details of the assays have been described previously^{8,9}. Ir-AVP was not detectable from fractions of N-IL or HT extracts prepared from the Brattleboro rats. The shaded area represents detection limit (0.5 pg per tube).

Although the chemical identity of the ir-AVP peaks found in the ovarian extracts of *di/di* and *LE/LE* rats has not been further established, the chromatographic profiles in Figs 1 and 2 strongly suggest the presence of nonapeptide AVP, in that activity co-elutes both with synthetic AVP (1-9) and with the ir-AVP peaks found in the gel profiles of HT and N-IL extracts from *LE/LE* rats. In these latter tissues, biologically active AVP (1-9) of molecular weight (M_r) $\sim$ 1,000 is the major end product, and the nonapeptide accounts for most of the ir-AVP-like activity^{1,10,11}. The absence of higher molecular weight ir-AVP species in both ovarian extracts may reflect an artefact of tissue handling; equally, it is consistent with rapid and extensive enzymatic processing of precursor(s) *in vivo*. Similarly, it is possible that the antigenic hexapeptide AVP (4-9) is shielded in the tertiary structure of the precursor(s), which thus may not be recognized by our antiserum. The absence of a high M_r peak of ir-AVP in *LE/LE* hypothalamic extracts is consistent with recent reports suggesting the early cleavage of AVP (1-9) from its $\sim$ 20,000 M_r precursor^{1,12,13}.

On a quantitative basis, the amount of ir-AVP in the ovary of both *di/di* and *LE/LE* rats is approximately equal; in both cases, the content is two to three orders of magnitude less than

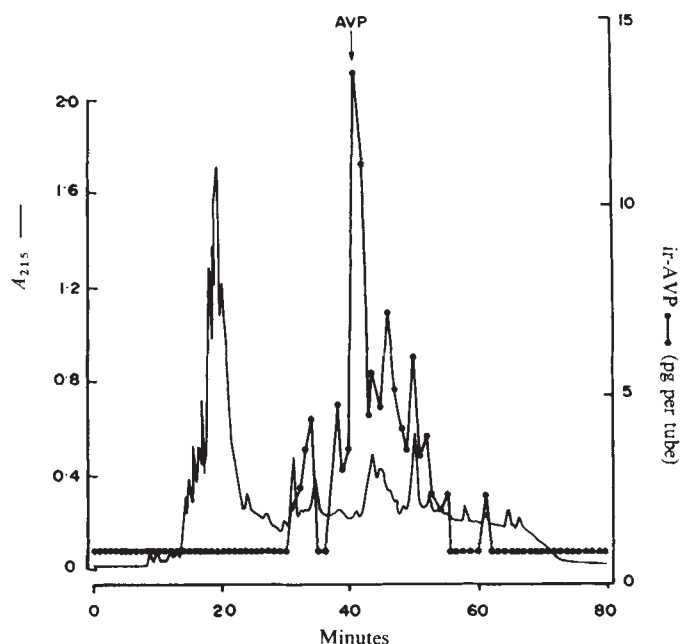


Fig. 2 HPLC profiles of ovarian extracts from homozygous Brattleboro (*di/di*) rats. Ovaries from 15 rats were collected in 10 ml of 0.1 M HCl at 4°C. Extracts were then prepared by homogenizing with a Polytron (Brinkmann Instruments, speed setting 3, 2 $\times$ 3-s bursts) and boiling for 15 min. Homogenates were centrifuged (10,000g, Sorvall RC-5, DuPont) for 20 min at 4°C. The supernatant was removed, lyophilized and reconstituted in 10 ml of 0.1% trifluoroacetic acid (TFA), applied to C18 Sep-pak cartridges, eluted at room temperature with 50% MeCN in 0.1% TFA and re-lyophilized for HPLC. Samples of rat ovarian extract (2 mg) were injected in 250 μ l of 0.1% TFA and gradients were run from 0% to 40% acetonitrile in 0.1% TFA over 60 min at 1.0 ml min⁻¹. HPLC was carried out using a Waters Associates system comprising a UK6 injector, two 6000A pumps, a 660 solvent programmer and a 441 UV absorbance detector at 215 nm. The column used was a Waters μ Bondapak C₁₈ radial-pak cartridge (10 μ m packing) pressurized to 110 kg cm⁻² in a RCM 100 compression module. Fractions were collected from 5 min before injection until 15 min after final gradient conditions were reached. Protein was estimated by absorbance at 215 nm (—), and fractions from the rat ovarian extract were lyophilized and assayed for immunoreactive AVP (●—●), with a calculated recovery of 85% of the applied ir-AVP. The arrow labelled AVP indicates the elution position of authentic AVP (Peninsula) run in identical conditions after the ovarian extract.

that present in the HT or N-IL. As such, ovarian ir-AVP seems unlikely to contribute substantially to circulating ir-AVP, but may act as a local modulator affecting ovarian function. Adashi and Hsueh¹³ have recently proposed a similar paracrine role for AVP in the male gonad, as they found that the peptide markedly suppressed testicular steroidogenesis in cell culture.

In immunofluorescence studies of adult ovaries from both *di/di* and *LE/LE* rats, positive staining for AVP was confined to the lutein cells of corpora lutea (Fig. 3a, b). In gonadotropin-treated animals, however, luteinized theca cells adjacent to the corpora lutea, and those scattered among the interstitial tissue, also stained positively for ir-AVP (Fig. 3c, d). In contrast, ovaries of immature rats or ovarian follicles of adult rats were immunofluorescence-negative for ir-AVP (data not shown). As expected, both ir-AVP (Fig. 3e) and ir-oxytocin (Fig. 3f) were localized immunohistochemically in the neurohypophysis of *LE/LE* rats; ir-oxytocin (Fig. 3g) but not ir-AVP (Fig. 3h) was identified in the same tissue of *di/di* rats.

As shown in Fig. 4a, ovarian ir-AVP seems to be related to the stage of sexual maturation of the animals. A 10-fold higher content of ir-AVP was found in ovaries of adult *di/di* and *LE/LE* rats than in those of immature (19–21 day) animals; in

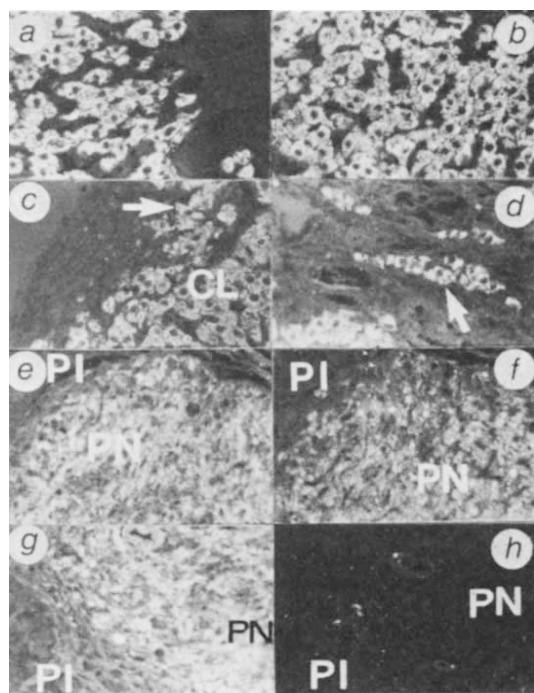


Fig. 3 Indirect immunofluorescence microscopy of pituitary and ovarian sections from Long-Evans (*LE/LE*) or homozygous (*di/di*) Brattleboro rats, showing positive staining for ir-AVP in the luteinized granulosa cells of the corpus luteum of adult Brattleboro rats treated with saline (*a*) or with pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (HCG) (*b*). In PMSG/HCG-treated animals, luteinized theca cells (arrows) adjacent to the corpus luteum (CL) are immunofluorescence-positive for ir-AVP (*c*), as are individual cells in the interstitium (*d*). Identical staining for ir-AVP was found in the ovary of adult *LE/LE* rats examined in parallel (data not shown). The neurohypophysis (PN) of *LE/LE* rats shows positive staining with anti-oxytocin (Immunonuclear) (*e*) and anti-AVP antiserum (R151) (*f*), with the intermediate lobe (PI) immunofluorescence-negative for both. In contrast, although the PN is positive for ir-oxytocin in *di/di* rats (*g*), both parts of the neurointermediate lobe are negative for ir-AVP (*h*). All tissues were fixed in Bouin's solution for 4 h at room temperature, dehydrated, cleared and paraffin infiltrated by a Autotechnicon Ultrapressor at 60 °C. Sections 3- μ m were prepared, delipidated, rehydrated and incubated with anti-AVP antiserum R151 (from 1:32 to 1:512) for 24 h at 4 °C. After 3 $\times$ 10-min washes in phosphate-buffered saline (PBS), fluoresceinated sheep anti-rabbit immunoglobulin (Wellcome) was added at a 1/20 dilution for 30 min at room temperature. The sections were rinsed and mounted for viewing under a Leitz-Dialux 20 microscope. Specificity of the staining reactions was demonstrated by the absence of specific staining with PBS, with normal rabbit serum, or with anti-AVP antiserum (R151 at 1:32) preabsorbed with an equal volume of 5 μ g ml⁻¹ synthetic arginine-vasopressin (Ferring). Staining was unaffected by prior absorption with oxytocin, Met-enkephalin, ovine β -endorphin, adrenocorticotrophic hormone or α -melanocyte-stimulating hormone at concentrations of 50–100 μ g ml⁻¹. Not shown is equivalent immunofluorescence staining obtained with an anti-AVP antiserum (23H2T, obtained from Immunonuclear, Stillwater, Minnesota).

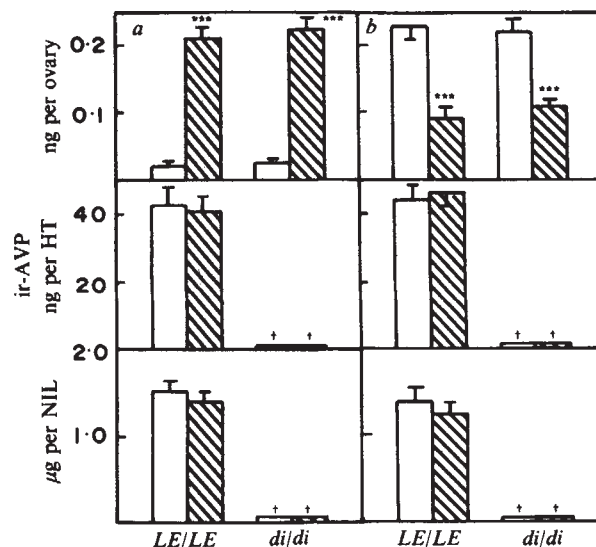


Fig. 4 Content of immunoreactive-arginine-vasopressin in ovary (upper panel), hypothalamus (HT; middle panel) and neurointermediate lobe (N-IL; lower panel) of Long-Evans rats (*LE/LE*; left) and homozygous Brattleboro rats (*di/di*; right). Rats were decapitated; hypothalamic tissue was defined rostrally by a cut 2 mm anterior to the optic chiasm, caudally by the mamillary bodies, and laterally by the lateral sulci, to a depth of 5 mm from the median eminence. Pituitaries were removed and separated into anterior pituitary and N-IL under a dissecting microscope; ovaries were similarly removed and cleared of fat. For radioimmunoassay, all the tissues were extracted by first boiling in 2 ml 0.1 M HCl for 15 min followed by homogenizing and centrifuging as described in Fig. 1 legend; supernatants so obtained were removed, freeze-dried, reconstituted and diluted with assay buffer to 1 in 1,000 (pituitary) and 1 in 100 (hypothalamus) for radioimmunoassay. *a*, Immature (19–22 days) rats (□) and mature rats (▨). *b*, Adult female rats (100–150 days) were injected intramuscularly once with 50 IU PMSG and 60 h later with 25 IU HCG (▨); control animals received saline injections on both occasions (□). All animals were killed 24 h after the last injection by decapitation. Values shown are mean $\pm$ s.e.m., $n=6$. *** $P<0.01$ compared with immature animals (*a*), or saline treated controls (*b*). † Not detectable (<0.5 pg per tube).

serum gonadotropin (PMSG) and human chorionic gonadotropin (HCG) lowered ovarian ir-AVP significantly in both *di/di* ($P<0.001$) and *LE/LE* rats ($P<0.001$), whereas ir-AVP contents of the HT or N-IL from the same animals remained unaltered. Such observations further support the contention that ovarian ir-AVP is specifically affected by the gonadotropins, independently of levels in intracranial tissues.

Accordingly, there appear to be several lines of evidence for an identical pattern of ovarian production of AVP in *LE/LE* and *di/di* rats, as has previously been suggested for the bovine⁷ and human¹⁴ ovary. As such, the absence of hypothalamic AVP in the *di/di* rat appears consistent with a local tissue-specific defect of gene transcription/translation. A transcriptional defect was postulated on the basis of dot-blot analysis of RNA from the supraoptic nuclei of homozygous Brattleboro and normal rats, using a specific AVP-NP11 probe². More recently¹⁵, the same workers have shown that a subtly altered (single nucleotide deletion in exon B) mRNA for AVP-NP11 from Brattleboro hypothalamus can be demonstrated, in normal amounts, from Brattleboro hypothalamus. Accordingly, they postulate that the disorder in Brattleboro hypothalamus is one of translation, rather than transcription, of the altered sequence. We are now investigating the control of expression of the gene coding for AVP in both hypothalamus and ovary, in an attempt to determine why it is expressed in the ovary but not the hypothalamus of the Brattleboro rat.

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contrast, ir-AVP content of HT and N-IL from both strains did not differ significantly with age. If there is a single copy of the AVP gene in the animals, our observations suggest that expression of the gene coding for AVP is quite differently controlled in ovary and brain. If there are multiple genes for AVP, our data suggest selective tissue-specific expression of one gene in HT, and another in ovary.

The marked differences in the ovarian contents of ir-AVP found between immature and adult rats prompted us to examine the possible effects of gonadotropins on ovarian peptide levels. Figure 4b shows that sequential treatment with pregnant mare

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1. Russell, J. T., Brownstein, M. J. & Gainer, H. *Endocrinology* **107**, 1880–1891 (1980).
2. Schmale, H., Heinsohn, S. & Richter, D. *EMBO J.* **2**, 763–767 (1983).
3. Lim, A. T. *et al. Nature* **303**, 709–711 (1983).
4. Sharp, B., Pekary, A. E., Meyer, N. V. & Hershan, J. W. *Biochem. biophys. Res. Commun.* **95**, 618–623 (1980).
5. Tsong, S. D. *et al. Endocrinology* **110**, 2204–2206 (1983).
6. Wathes, D. C. & Swann, R. W. *Nature* **297**, 225–227 (1982).
7. Wathes, D. C., Swann, R. W., Birkett, S. D., Porter, D. G. & Pickering, B. T. *Endocrinology* **113**, 693–698 (1983).
8. Pullan, P. T., Johnston, C. I., Anderson, W. P. & Korner, P. I. *Clin. Sci. molec. Med.* **55**, 251s–254s (1978).
9. Wood, R. L. & Johnston, C. I. *Am. J. Physiol.* **363**, F727–F732 (1982).
10. Gainer, H., Sarne, Y. & Brownstein, M. J. *J. Cell Biol.* **73**, 366–381 (1977).
11. Russell, J. T., Brownstein, M. J. & Gainer, H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6086–6090 (1979).
12. Land, H., Schutz, G., Schmale, H. & Richter, D. *Nature* **295**, 299–303 (1982).
13. Adashi, E. Y. & Hsueh, A. J. W. *Endocrinology* **109**, 1793–1795 (1981).
14. Wathes, D. C. *et al. Lancet* **ii**, 410–412 (1982).
15. Schmale, H. & Richter, D. *Nature* **308**, 705–709 (1984).

Brattleboro rat adrenal contains vasopressin

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Arginine-vasopressin (AVP) is a neurohypophysial nonapeptide with antidiuretic activity involved in the control of blood volume and plasma osmolality¹. Recently, by immunological methods, the presence of AVP has been demonstrated in extrahypothalamic areas of the brain, in the spinal cord² and in the ovary³, testis⁴ and adrenal gland⁵. The Brattleboro rat is regarded as having an autosomal recessively inherited lack of neurohypophysial vasopressin⁶ and its associated neurophysin⁷. Since its discovery over 20 years ago⁸ this animal has been widely used in studies on the physiological role of vasopressin. We have recently investigated the presence of immunoreactive vasopressin and the related non-peptide oxytocin in the adrenal glands of the human, rat and cow, and report here the isolation from the Brattleboro rat adrenal of material with similar immunological, physical and biological properties to synthetic vasopressin.

Groups of female Brattleboro rats (200 g) were obtained from the Charing Cross Hospital Medical School; these animals were the products of homozygote–homozygote matings and were confirmed to have diabetes insipidus (urine output > 140 ml per day). Female Long–Evans rats of the same weight and source were used as controls. Vasopressin and oxytocin were measured using highly specific radioimmunoassays (RIAs)², the First International AVP Standard (77/501) and the Fourth International Oxytocin Standard (76/575). The cross-reactivity between these hormone assays was <0.005%. Animals were allowed free access to water and were killed by stunning and decapitation.

Trunk blood was collected and hypothalami, pituitary and adrenal glands were snap-frozen after their rapid removal; they were stored at –70 °C until used. Extraction of plasma, pituitary and hypothalamic AVP was performed as described previously².

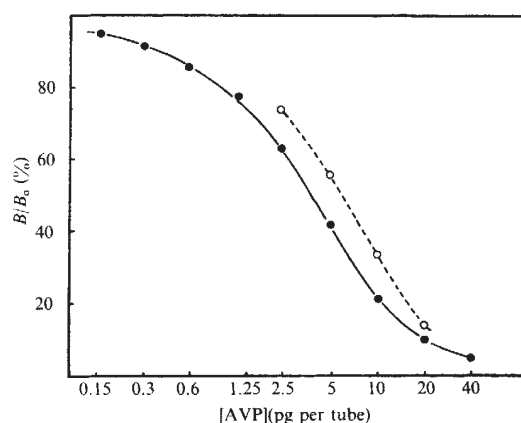


Fig. 1. Immunoreactivity of serial dilutions of Brattleboro rat adrenal extract. Brattleboro rat adrenal extract (○) was prepared by HPLC in conditions described in Fig. 3 legend. Serial dilutions of this material were made and compared with serial dilutions of the AVP standard (●) by RIA².

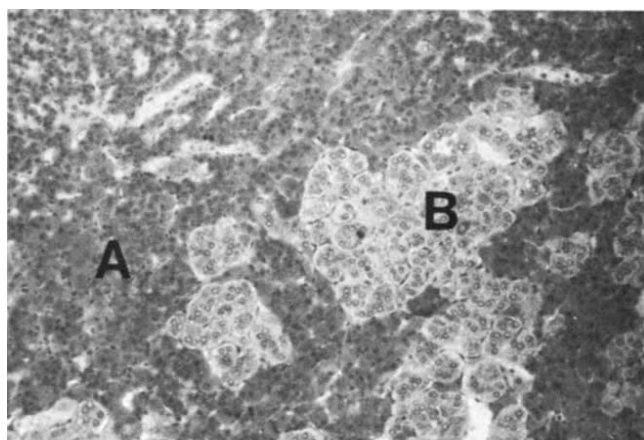


Fig. 2. Immunocytochemical localization of AVP, using Bouin-fixed Brattleboro rat adrenal gland, anti-AVP antiserum (at 1:150 dilution) and peroxidase–antiperoxidase. Sections (7 μm) were taken through the centre of the gland. The counterstain was Mayer's haemalum and control sections were incubated with preabsorbed antiserum. The photomicrograph (×62), from the centre of the medulla, shows darker, stained cells (A) with lighter, negative cells (B).

Table 1 Immunoreactive neurohypophysial peptides in Brattleboro and Long–Evans rats

Tissue	Rat strain	AVP	Oxytocin
Plasma	Long–Evans	8.0 ± 6.5 pg ml ⁻¹ (n = 11)	17.0 ± 12.0 pg ml ⁻¹ (n = 11)
	Brattleboro	* <0.5 pg ml ⁻¹ (n = 7)	87.6 ± 75.0 pg ml ⁻¹ (n = 15)
Adrenal gland	Long–Evans	11.5 ng g ⁻¹ (pool of 12)	12 ng g ⁻¹ (pool of 12)
	Brattleboro	14.5 ng g ⁻¹ (pool of 12)	16 ng g ⁻¹ (pool of 12)
Hypothalamus	Long–Evans	1.58 ± 0.28 μg g ⁻¹ (n = 4)	0.46 ± 0.25 μg g ⁻¹ (n = 4)
	Brattleboro	0.24 ng g ⁻¹ (pool of 6)	93 ng g ⁻¹ (pool of 6)
Pituitary	Long–Evans	130 ± 22 μg g ⁻¹ (n = 3)	56 ± 32 μg g ⁻¹ (n = 3)
	Brattleboro	0.54 ng g ⁻¹ (pool of 6)	71 μg g ⁻¹ (pool of 6)

The plasma and tissue content of immunoreactive AVP and oxytocin was compared in the Brattleboro and Long–Evans rats. RIAs were performed on either tissue extracts from individual animals (numbers in parentheses) or, particularly in the case of the Brattleboro rat where concentrations were low, an extract from pooled glands. Values are the mean ± s.d.

* 0.84 ± 0.2 pg ml⁻¹ in a further five animals.

RIAs showed similar concentrations of AVP in the adrenals of Brattleboro and Long-Evans rats but markedly reduced amounts in Brattleboro plasma, pituitary and hypothalamus (Table 1). Oxytocin concentrations were approximately the same in the adrenals of both strains but higher in Brattleboro plasma and lower in Brattleboro rat hypothalamus.

We confirmed the presence of AVP in the Brattleboro adrenal by showing that serial dilutions gave a curve parallel to the standard curve of the reference peptide in RIA (Fig. 1). In addition, immunocytochemistry using a different AVP antibody

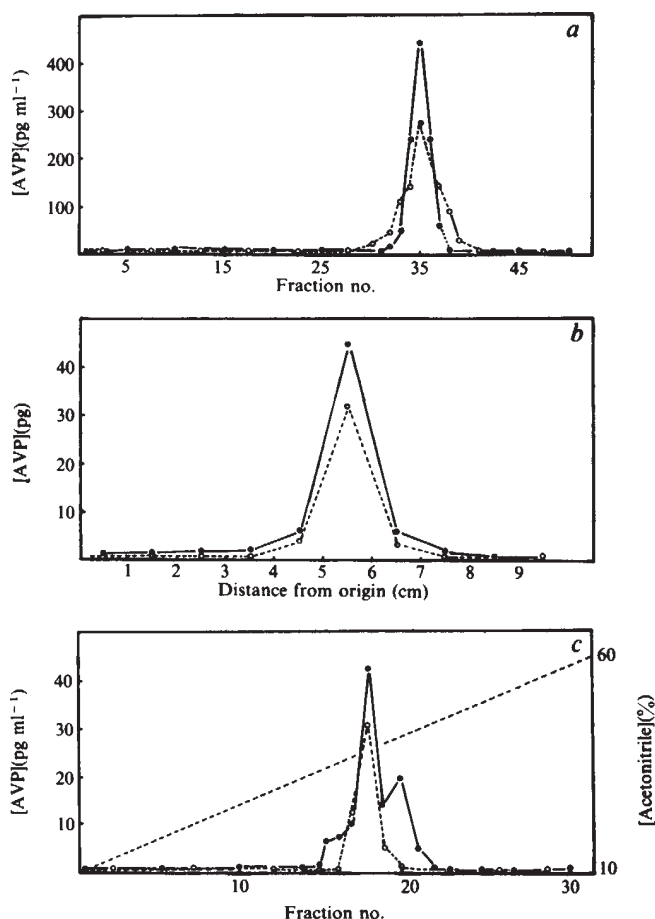


Fig. 3 Chromatography of Brattleboro rat adrenal extracts. Tissues were homogenized in 1 M acetic acid (300 mg tissue per ml) using five strokes of a glass-glass homogenizer. The homogenate was centrifuged in an Eppendorf microfuge for 5 min and the supernatant removed and subjected to chromatography. Synthetic vasopressin (Ferring) was subjected to the same procedures in identical conditions and the immunoactivity profiles were compared. ●, Brattleboro rat adrenal extract; ○, synthetic AVP. *a*, 500 µl of Brattleboro rat adrenal extract were chromatographed on a Biogel P2 (Biorad) 1 × 60 cm column eluted at 10 ml h⁻¹ with 0.1 M formic acid. Fractions (1 ml) were collected and 100-µl aliquots diluted with 100 µl 0.2 M Tris-HCl pH 8.0, then assayed for AVP. Synthetic AVP (2 ng) was run for comparison. Blue dextran 2000 eluted in fraction 17. *b*, 100 µl of Brattleboro rat adrenal extract were mixed with 100 µl of acetone and centrifuged. The supernatant was spotted on a Whatman LK6DF TLC plate and run in *n*-butanol acetic acid pyridine water (15:3:10:6) 1-cm lengths of the plate were scraped and eluted with 60% acetonitrile, dried down and assayed after resuspension in assay buffer. Synthetic AVP (1 ng) was run on the same plate. The *R_f* of synthetic AVP in this system was 0.454. *c*, 50 µl of Brattleboro rat adrenal extract were chromatographed on a Waters Associates instrument with a µ-Bondapak C₁₈ column, with a 10–60% linear gradient of acetonitrile in water containing 0.08% trifluoroacetic acid, at a flow rate of 1 ml min⁻¹. Fractions (1 ml) were collected, dried down and assayed for AVP after resuspension in assay buffer.

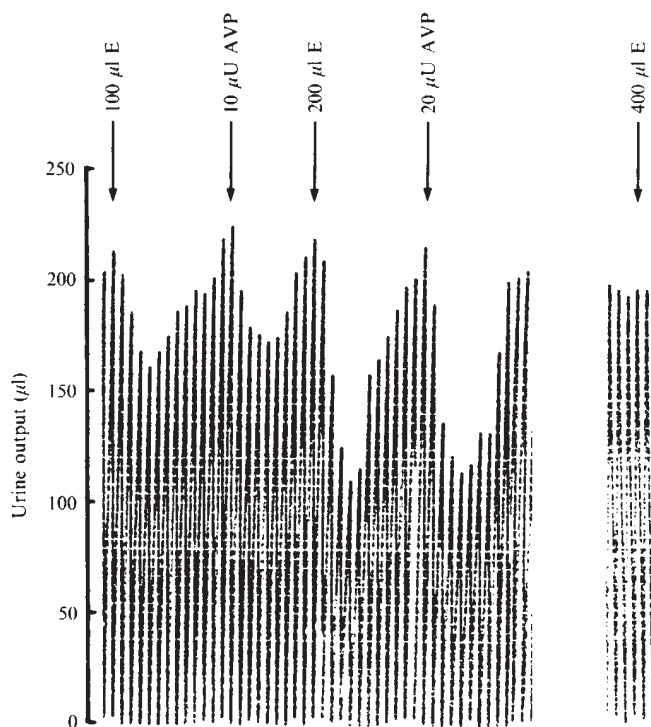


Fig. 4 Bioassay of antidiuretic activity. Antidiuretic activity was assayed in the water-loaded, ethanol-anaesthetized rat⁹. Each vertical line represents the urine output in 1 min. Intravenous injections were given at the arrows. E, extract (1 ml) of HPLC-purified immunoreactive material from three pairs of Brattleboro rat adrenals. AVP, vasopressin (Pitressin). After injection of 20 µg of d(CH₂)₅Tyr(Et)VAVP a specific AVP antagonist (VAVP is 4-value arginine vasopressin)¹⁰, during the interval between the tracings, 400 µl of extract produced no antidiuretic response.

demonstrated immunoreactive material in the adrenal medulla (Fig. 2). However, further immunocytochemical studies (not shown) have demonstrated reactivity in the zona fasciculata and reticularis but not in the zona glomerulosa of the adrenal cortex.

Immunoreactive AVP extracted from Brattleboro rat adrenals behaved identically to synthetic AVP on gel chromatography, TLC and HPLC (Fig. 3). Conditions were chosen to give a clear separation from OXT. The small additional immunoreactive peak with a retention time of 20 min on HPLC was not present in all experiments and was also seen in material from Long-Evans rats.

Antidiuretic activity was demonstrated⁹ in four different extracts of purified immunoreactive material from the adrenal glands of Brattleboro rats. In the experiment of Fig. 4, intravenous injections of the extract and of vasopressin (Pitressin) produced graded antidiuretic responses with similar profiles and the antidiuretic activity of the extract was abolished by a specific AVP antagonist¹⁰. The total antidiuretic activity of the extract was ~100 µU; the amount of immunoreactive material was equivalent to 200 pg of the AVP standard, giving ~500 IU of biological activity per mg of immunoassayable material.

It has been shown previously in our laboratory that the adrenal glands of several species contain material which is immunologically and chromatographically identical with synthetic AVP and oxytocin⁵. The present work extends these studies and shows that the adrenal gland of the Brattleboro rat contains an AVP-like material in amounts equal to those of the normal animal. This material behaves identically to synthetic vasopressin on gel chromatography, TLC and HPLC; it is recognized by antibodies to vasopressin and has antidiuretic activity which is abolished by a specific AVP antagonist. We consider it highly likely, therefore, that the material is vasopressin. In contrast, we were

able to detect immunoreactive material in the Brattleboro hypothalamus, pituitary gland and plasma only at very low concentrations, which were near the limits of the assay, and we were unable to detect biologically active AVP in Brattleboro pituitary extracts.

We believe these results have implications for the interpretation of the results of physiological experiments in the Brattleboro rat, a rat strain long considered to be AVP-deficient. In particular, they highlight the possible roles of both oxytocin and AVP in catecholamine and glucocorticoid synthesis and secretion. In addition, our results may be relevant to the understanding of AVP gene regulation.

Since the completion of this study, Schmale and Richter have published results of elegant studies on the Brattleboro rat¹¹.

Received 2 April; accepted 25 May 1984.

1. Moses, A. M. & Miller, M. *Handbook of Physiology*, Section 7 Vol. 4, Pt 1, 225-242 (American Physiological Society, 1974).
2. Jenkins, J. S., Ang, V. T. Y., Hawthorn, J., Rosser, M. N. & Iversen, L. L. *Brain Res.* **291**, 111-117 (1984).
3. Wathes, D. C., Swann, R. W., Birkett, S. D., Porter, D. G. & Pickering, B. T. *Endocrinology* **113**, 693-698 (1983).

They have identified the defect in the AVP gene as a single deletion in the sequence encoding the neurophysin and postulate subsequent abnormalities in translation and post-translational events. The presence of AVP in the Brattleboro rat adrenal suggests that this tissue, unlike the hypothalamus, may be capable of post-translational modification of the abnormal precursor. However, the possibility of a second AVP gene expressed in the Brattleboro rat adrenal is not excluded.

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4. Nicholson, H. D. *et al. Acta endocr.* **103** (Suppl. 256), 243 (1983).
5. Ang, V. T. Y. & Jenkins, J. S. *J. clin. Endocr.* **58**, 688-691 (1984).
6. Valtin, H. & Schroeder, H. A. *Am. J. Physiol.* **206**, 425-430 (1964).
7. Burford, G. D., Jones, C. W. & Pickering, B. T. *Biochem. J.* **124**, 809-813 (1971).
8. Valtin, H., Schroeder, H. A., Benirschke, K. & Sokol, H. W. *Nature* **196**, 1109-1110 (1962).
9. Bisset, G. W. & Chowdrey, H. S. *J. Physiol., Lond.* (in the press).
10. Sawyer, W. H. *et al. Science* **212**, 49-51 (1981).
11. Schmale, H. & Richter, D. *Nature* **308**, 705-709 (1984).

Inviability of parthenogenones is determined by pronuclei, not egg cytoplasm

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Parthenogenetic mouse embryos pose an interesting problem in the study of early mammalian development. Haploid or diploid parthenogenones, resulting from spontaneous¹ or experimental²⁻⁴ activation of unfertilized eggs, will undergo apparently normal preimplantation development but die in the early post-implantation stages⁵⁻⁸. However, in aggregation chimaeras with fertilized embryos, parthenogenetic embryos have the ability to differentiate into many tissue types, including gametes which can give rise to normal offspring⁹. Furthermore, it has been reported that viable young were obtained from the transfer of inner cell-mass nuclei of parthenogenetic blastocysts to enucleated fertilized eggs¹⁰. These observations suggest that sperm have some additional role, apart from restoring a complete genome, that is necessary for normal development. To investigate whether sperm-related modifications to the egg cytoplasm are important, we have used an efficient nuclear transfer technique in which a complete karyoplast, comprised of pronuclei, surrounding cytoplasm and a portion of the egg plasma membrane, is transferred utilizing Sendai virus membrane fusion¹¹. Embryos produced by the transfer of pronuclei from diploid parthenogenetic eggs to enucleated fertilized eggs died very soon after implantation, whereas viable young were obtained from the transfer of fertilized egg pronuclei into enucleated parthenogenetic eggs. This shows that the death of parthenogenones is not due to a lack of cytoplasmic factors from the sperm.

In the experiments of McGrath and Solter¹¹, 15% of the enucleated eggs receiving transferred karyoplasts developed successfully to term. Our preliminary experiments showed a higher success rate, 66%, but this can be attributed to more efficient implantation. Nuclei from fertilized eggs were transferred to enucleated fertilized eggs of contrasting genetic type, and the resulting embryos replaced in pseudopregnant females. One recipient failed to become pregnant; the remaining females received 15 manipulated embryos and gave birth to 10 live young, identified by enzyme type or coat colour as derived from the donor nuclei.

Nuclei from fertilized eggs were then transferred to parthenogenetically activated and enucleated host eggs (Table 1). Of 32 manipulated embryos put into females that became pregnant, 22 gave rise to live young, all of which were identified

as being derived from donor nuclei; 4 by enzyme type and 18 by pigmentation. Thus, karyoplasts transferred to enucleated eggs supported successful development through to birth in at least 66% of cases, irrespective of whether the host eggs were fertilized or parthenogenetically activated before enucleation.

In contrast, when nuclei from parthenogenetically activated eggs were transferred into enucleated fertilized eggs (Table 2), none of the manipulated embryos developed to term. In one experiment (data not shown) 5 out of 6 manipulated embryos of this class were allowed to develop to blastocysts *in vitro*. These appeared normal and underwent hatching. Of 34 put into females that became pregnant, 21 implanted successfully, but all 21 were retarded or degenerating when the recipient females were killed 6½-10½ days post coitum. In recipients at 10½ and 7½ days, only a small amount of necrotic tissue was apparent, while in recipients autopsied at 6½ days of pregnancy, the experimental embryos were retarded in growth and disorganized (only two had formed recognizable egg cylinders). Except in one case, embryos in the control horn were always morphologically normal and at an expected stage of development. Evidently the parthenogenetic karyoplasts were not capable of supporting normal development.

These results strongly argue against the possibility that the cytoplasm of fertilized eggs has components which could support those aspects of normal development not achieved in

Table 1 Transfer of fertilized egg pronuclei into enucleated parthenogenetic eggs

Recipient female	Donor nuclei	Host eggs	No. of embryos: Replaced	Born	Phenotype of young	Sex of young
1	GPI-IAB	GPI-IB	4	4	GPI-IAB	4♂
2	c/c	+/+	13	10	c/c	3♀, 6♂
3	c/c	+/+	10	5	c/c	1♀, 2♂
4	c/c	+/+	5	3	c/c	1♀, 2♂
Total			32	22		

Recipient females, and females used to provide parthenogenetic eggs, were (CBA × C57BL/6J)F₁ animals, homozygous for non-albino and for the electrophoretically distinguishable B variant of GPI-I (glucose phosphate isomerase 1); donor nuclei were either from heterozygous GPI-IAB eggs of the randomly bred Q strain (recipient 1) or from homozygous albino eggs of the randomly bred MFI strain (recipients 2, 3, 4). Oocytes and fertilized eggs were obtained from mice induced to ovulate by injection of 5 IU pregnant mare serum followed 48 h later by 5 IU human chorionic gonadotropin, and were manipulated in medium M2²¹. Oocytes were activated parthenogenetically by ethanol treatment^{3,4}. Those of the haploid class (2nd polar body, 1 pronucleus) were enucleated, and pronuclei of fertilized eggs were then transferred to them¹¹. After culture overnight in medium 16²² at 37 °C, in 5% CO₂ in air and a humidified atmosphere, all 43 successfully manipulated eggs cleaved to two cells. In recipients 1 and 4, embryos were put into a uterine horn at the blastocyst or compacted morula stage on the 3rd day of pseudopregnancy. In recipients 2 and 3, embryos were put into oviducts at the 2-cell stage on the 1st day of pseudopregnancy. Recipients were mated to vasectomized males of the same strain and of proven sterility. One recipient failed to become pregnant. Of the young born, three died before sexing; all others survived to adulthood, and the four males from recipient 1 have bred.

Table 2 Transfer of parthenogenetic nuclei into enucleated fertilized eggs

Recipient female	Autopsy (days post coitum)	No. of embryos					
		Control		Experimental			
		R	N	A	R	N	A
1	10½	0	—	—	8	0	2
2	10½	6	5	1	3	0	3
3	10½	3	2	0	3	0	2
4	7½	5	5	0	5	0	5
5	6½	5	5	0	5	0	4
6	6½	4	4	0	5	0	0
7	6½	6	0	0	5	0	5
Total		29	21	1	34	0	21

R, replaced; N, normal; A, abnormal.

F₁ animals (see Table 1) were used to provide recipient females 4, 5 and 6, donor nuclei for the transfers in 1, 2, 3, 5, 6 and 7, and host eggs in 4, 5, 6 and 7. In other cases MF1 animals were used. Parthenogenetically activated oocytes of the diploid class (2 pronuclei, no 2nd polar body) were selected (recipients 1–3) or obtained by culture for 2½ h in cytochalasin B to suppress 2nd polar body formation, followed by 4 h culture in medium 16 without cytochalasin B (recipients 4–7). For other technical details, see note to Table 1. Of 44 embryos successfully manipulated, 41 cleaved to two cells. Experimental embryos were put into one oviduct of a pseudopregnant recipient (both oviducts for recipient 1), whereas control two-cell embryos (fertilized embryos of the same type used as host eggs recovered and cultured along with the experimentals, but not otherwise manipulated) were put into the other oviduct. Two recipients failed to become pregnant.

parthenogenetic eggs. Indeed they suggest that it is some property of the paternal pronucleus that is essential, or perhaps some perinuclear component inseparable by this technique. A similar conclusion can be drawn from the recent finding of Surani and Barton¹² and others^{13,14}, in which diploid gynogenetic eggs (fertilized eggs containing two maternal but no paternal pronuclei) do not develop beyond mid-gestation. It is difficult to reconcile our results with those of Hoppe and Illmensee¹⁰, showing development to term upon transfer of parthenogenetic ICM cell nuclei to enucleated fertilized eggs. However, it is conceivable that some modification occurs to the nuclear material during development to the blastocyst stage. Another explanation of these conflicting results could lie in the differences in technique used for enucleation. In the method of McGrath and Solter¹¹ used in this study, all of the pronucleus is unequivocally removed as it is sucked into the enucleation pipette surrounded by the egg plasma membrane. However, with the method used by Hoppe and Illmensee, which involves puncture of the egg membrane, part of the pronucleus may be left behind during enucleation. It is possible that this remaining material was sufficient to permit development to term. This may also provide an explanation for the differences between the results obtained by Hoppe and Illmensee¹⁵ and others^{12–14} with diploid gynogenetic eggs.

The cause of death of parthenogenones remains unknown. We do not consider that homozygosity plays a role as has been proposed^{12,16}, since the number of lethal mutations necessary to bring about the death of every parthenogenone would require an unrealistically high mortality in brother–sister matings of inbred strains. The essential role of the paternal pronucleus may involve one of the nuclear components known to differ between sperm and egg, such as protamines¹⁷; alternatively, specific modifications to sperm DNA, such as methylation, may be important. Such differences may also be reflected functionally at a later stage in the preferential inactivation of the paternal X chromosome in the extra-embryonic membranes^{18,19}.

Since submission of this paper, work by Surani *et al.*²⁰ has been published that reaches similar conclusions. By transferring single pronuclei from fertilized eggs to haploid parthenogenones they demonstrated that the paternal pronucleus is necessary for normal development.

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Note added in proof: By transferring single pronuclei between fertilized eggs McGrath and Solter²³ have shown that the combination of paternal and maternal pronuclei is essential.

Received 14 March; accepted 15 May 1984.

1. Stevens, L. C. & Varnum, D. S. *Devl Biol.* **37**, 369–380 (1974).
2. Kaufman, M. H. in *Methods in Mammalian Reproduction* (ed. Daniel, J. C.) 21–47 (Academic, London, 1978).
3. Kaufman, M. H. *J. Embryol. exp. Morph.* **71**, 139–154 (1982).
4. Cuthbertson, K. S. R., Whittingham, D. G. & Cobbold, P. H. *Nature* **294**, 754–757 (1981).
5. Witkowska, A. *J. Embryol. exp. Morph.* **30**, 519–545 (1973).
6. Witkowska, A. *J. Embryol. exp. Morph.* **30**, 547–560 (1973).
7. Kaufman, M. H., Barton, S. C. & Surani, M. A. H. *Nature* **265**, 53–55 (1977).
8. Kaufman, M. H. *J. Embryol. exp. Morph.* **45**, 85–91 (1978).
9. Stevens, L. C. *Nature* **276**, 266–267 (1978).
10. Hoppe, P. C. & Illmensee, K. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1912–1916 (1982).
11. McGrath, J. & Solter, D. *Science* **220**, 1300–1302 (1983).
12. Surani, M. A. H. & Barton, S. C. *Science* **222**, 1034–1036 (1983).
13. Modlinski, J. A. *J. Embryol. exp. Morph.* **60**, 153–161 (1980).
14. Markert, C. L. *J. Hered.* **73**, 390–397 (1982).
15. Hoppe, P. C. & Illmensee, K. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5657–5661 (1977).
16. Graham, C. F. *Biol. Rev.* **49**, 399–422 (1974).
17. Balhorn, R. *J. Cell Biol.* **93**, 298–305 (1982).
18. Takagi, N. & Sasaki, M. *Nature* **256**, 640–642 (1975).
19. Rastan, S., Kaufman, M. H., Handyside, A. H. & Lyon, M. F. *Nature* **288**, 172–173 (1980).
20. Surani, M. A. H., Barton, S. C. & Norris, M. L. *Nature* **308**, 548–550 (1984).
21. Fulton, B. P. & Whittingham, D. G. *Nature* **273**, 149–151 (1978).
22. Whittingham, D. G. *J. Reprod. Fert. Suppl.* **14**, 7–21 (1971).
23. McGrath, J. & Solter, D. *Cell* **37**, 179–183 (1984).

Mechanism of sequential induction of cell-type specific mRNAs in *Dictyostelium* differentiation

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Upon starvation, the cellular slime mould *Dictyostelium discoideum* initiates a 24-h programme of differentiation¹. Within 6 h, cells move towards aggregation centres in response to pulsatile synthesis and secretion of cyclic AMP^{2–4}. At about 12 h, aggregates of 10⁵ cells are formed, held together by newly made surface adhesion molecules^{5–8}. The cells then differentiate into the two principal types found in the terminal stage of development, spores and stalks. Here we show that the chemotaxis and aggregation stages of this developmental programme can be described as a series of sequential events in which these extracellular signals—starvation, cyclic AMP and cell–cell contact—induce specific, sequential changes in the pattern of gene expression.

Among the gene products induced by each extracellular signal are cell-surface molecules which may enable the cells to recognize the signals that trigger subsequent phases of the programme. We have previously cloned and characterized a series of cDNAs which represent developmentally regulated genes preferentially expressed in pre-spore or pre-stalk cells⁹. The mRNAs encoded by these clones fall into four discrete classes, two specifically expressed in pre-spore cells and two in pre-stalk cells. Figure 1a shows the accumulation of mRNAs representative of each class. Pre-stalk class I mRNAs, such as D14, are present at very low levels in growing cells (see Fig. 1 of ref. 9) but accumulate to 20–50-fold higher levels by 12 h. Pre-stalk class II mRNAs, represented by PL1, are absent in growing cells and begin to accumulate between 8 and 12 h, at the time of chemotaxis. Both classes of pre-spore mRNAs are undetectable in growing cells; pre-spore I mRNAs (D18) begin to accumulate during chemotaxis (4–8 h), while the pre-spore II messages are first seen at the time tight cell contacts are formed (12 h). Mehdy *et al.*¹⁰ have recently cloned pre-stalk mRNAs which appear similar to our pre-stalk class II, and pre-spore-specific mRNAs similar to our class II pre-spore genes.

When growing cells are transferred to starvation buffer and shaken at 250 r.p.m., the initial stages of differentiation are induced, but cyclic AMP signalling is disrupted and the formation of cellular aggregates prevented. In such conditions, pre-stalk I mRNAs are induced with the same time course and to

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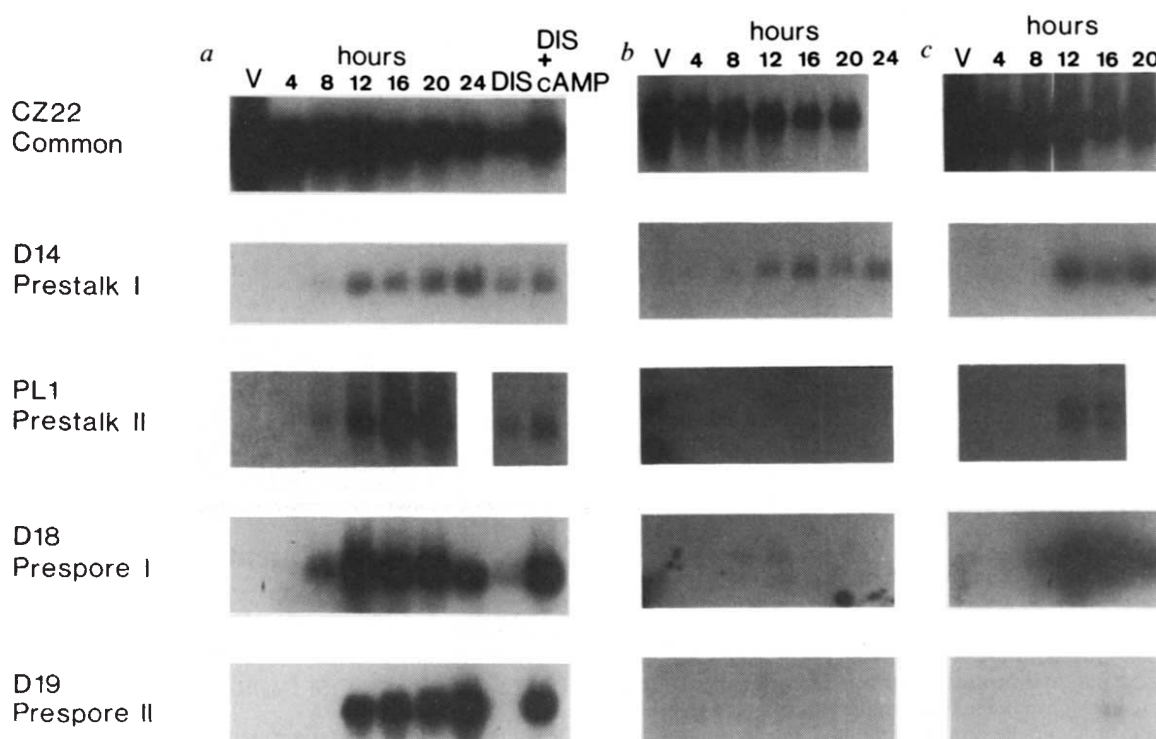
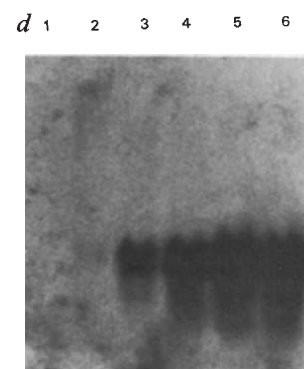


Fig. 1 Accumulation of pre-spore- and pre-stalk-specific mRNAs during normal development (*a*) and as a result of starvation in suspension (*b*) and cyclic AMP pulses (*c*), and induction of D19 (pre-spore II) mRNA following cell contact (*d*).

Methods: Total cytoplasmic RNA was prepared from developing cells at the times indicated¹⁷. RNA (25 µg) was size fractionated by electrophoresis on formaldehyde agarose gels, blotted to Gene Screen membranes and probed with ³²P-labelled cloned DNAs as described previously⁹. To facilitate comparisons, the filters probed with a given cloned message were hybridized in the same solution and autoradiographed on the same film; thus, for example, the level of D18 at 12 h of normal development (*a*) can be directly compared with the level at 12 h in starved suspension culture (*b*). *a*, *Dictyostelium* strain Ax3 cells were grown and plated for development on filter pads as previously described¹⁷. Cell aggregation is complete by 12 h and fruiting bodies form at 24–26 h of development. The lanes labelled DIS and DIS+cAMP contain total cytoplasmic RNA prepared from cells disaggregated after 15 h of development, then shaken for 4 h in the absence (DIS) or presence (DIS+cAMP) of 100 µM cyclic AMP. *b*, Growing cells were collected at 2×10^6 per ml, washed and resuspended at 1×10^7 per ml in PDF buffer¹⁷, and shaken at 22 °C and 250 r.p.m. on a gyratory shaker for the times indicated. *c*, Cells were collected and placed in starved suspension cultures as described for *b*. Starting at 2 h of starvation, cells were exposed to 50 nM pulses of cyclic AMP every 6 min. *d*, Cells were starved in suspension and pulsed with cyclic AMP as in *c*. At 12 h of development they were collected and 7.5×10^8 cells were deposited on a 132-mm filter pad. RNA was prepared from cells at the time of plating (track 1) and after 1, 2, 3, 4 or 8 h of further incubation (tracks 2–6, respectively).



levels similar to those seen during normal development (Fig. 1*b*). The other three classes do not accumulate to significant levels, although extremely low levels of D18 (pre-spore I) are occasionally seen. The levels of 'common' RNAs, such as CZ22, that are present throughout growth and differentiation, are unchanged. Thus, starvation alone is sufficient to induce the accumulation of pre-stalk I mRNAs to levels seen during normal development. Figure 2 shows that the cell-surface cyclic AMP receptor is also induced to normal levels in starved suspension cultures, but there is no induction of the developmentally regulated cell-surface molecules that allow formation of the EDTA-resistant aggregates (Fig. 3) characteristic of differentiating cells¹¹. Starvation can be viewed as an extracellular signal that induces a new pattern of gene expression, including induction of pre-stalk I mRNAs and the cell-surface cyclic AMP receptor.

Pulsatile addition of cyclic AMP to cells shaken in starvation buffer mimics the cell-cell signalling that occurs during normal aggregation. Figure 1*c* shows that in the presence of cyclic AMP pulses, the pre-stalk I and common mRNAs accumulate to the same extent as in starved suspension cultures without exogenous cyclic AMP. Importantly, cyclic AMP pulses induce class II

pre-stalk messages (PL1) and pre-spore I mRNAs (D18) to levels similar to those seen during normal development, and induce the ability of the cells to form EDTA-resistant aggregates (Fig. 3). Thus, cyclic AMP pulses represent another extracellular signal that induces a later stage in the developmental programme.

Pre-spore II mRNAs do not accumulate in cyclic AMP-pulsed, starved suspension cultures (Fig. 1*c*). However, if cells which have been pulsed for 12 h are placed on filters, the cells aggregate within 60 min. The pre-spore II messages begin to accumulate within 60 min of plating (Fig. 1*d*). Similarly, if cells pulsed with cyclic AMP for 12 h are subsequently shaken at low speeds (40 r.p.m. on a roller drum) where large aggregates are formed rapidly, induction of pre-spore II mRNAs is also seen (data not shown), although to somewhat lower levels. Presumably, induction of pre-spore II genes in roller culture is less dramatic because a smaller fraction of the cells are actually in aggregates. These results show that cell contact or some other consequence of aggregate formation triggers progression into the next stage of development, the expression of the large class of previously unexpressed pre-spore II genes.

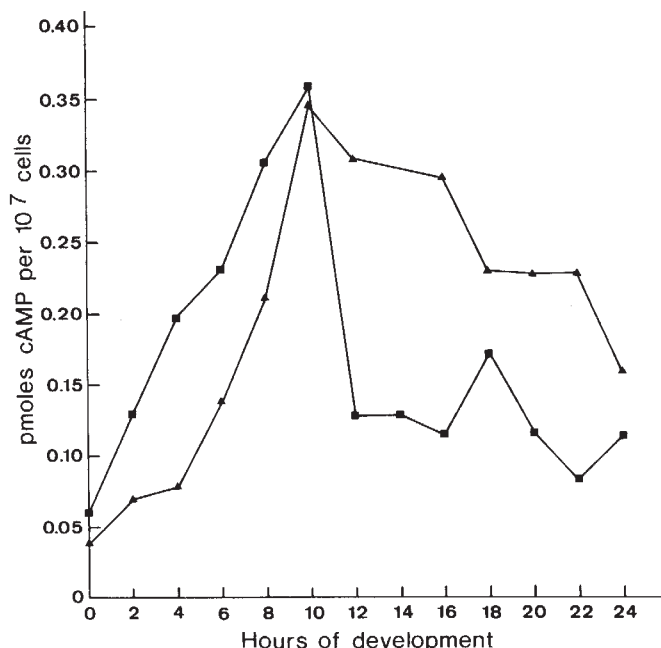


Fig. 2 Kinetics of appearance of cell-surface cyclic AMP binding activity. Cells were collected at the indicated times from filter pads (squares) or from starved suspension cultures (triangles), washed with PDF and resuspended at 1×10^8 cells per ml in PDF containing 10 mM dithiothreitol. 300 μ l of cells were mixed with 300 μ l of PDF containing 10^{-7} M 3 H-cyclic AMP, and incubated for 30 s at 4°C. Then 300 μ l of the mixture was layered on to 100 μ l of an 8:2 mixture of DC550 silicon oil and Nujol mineral oil. Following a 2-s centrifugation in an Eppendorf minifuge, the tubes were frozen, the bottom of the tube was cut off, and the pellet solubilized in 1% SDS. Specific cyclic AMP binding was calculated as the difference between the c.p.m. bound in the absence and presence of 10^{-4} M unlabelled cyclic AMP.

Figure 4 represents our current view of the *D. discoideum* developmental programme. It is a sequence of events in which extracellular signals trigger changes in the patterns of gene expression. At each stage new cell-surface molecules appear which could act as mediators for the effects of a subsequent extracellular signal. Starvation induces increased accumulation of pre-stalk I genes and the cell-surface cyclic AMP receptor. Pulses of extracellular cyclic AMP then result in induction of pre-stalk II and pre-spore I messages, as well as the ability to form EDTA-resistant cell aggregates. Finally, formation of aggregates results in the expression of pre-spore II mRNAs, the largest class of developmentally regulated genes.

Removal of the signal responsible for progression from one developmental stage to the next results in loss of gene products specifically induced at that stage^{12,13}. Figure 1a shows that disruption of the multicellular aggregates after 15 h of development results in the loss of messages encoded by pre-spore I, pre-spore II and pre-stalk II gene classes. Presumably, disruption of these cell aggregates eliminates both the aggregate-dependent signal responsible for pre-spore II gene expression and the cyclic AMP signal necessary for induction of pre-stalk II and pre-spore I genes. Expression of pre-stalk I genes, induced by starvation, is unaffected by disaggregation. Moreover, addition of 100 μ M cyclic AMP to disaggregated cells results in both renewed transcription of pre-spore I and pre-stalk II gene classes and stabilization of their cytoplasmic mRNAs^{13,14}. Interestingly, accumulation of pre-spore II messages is also restored by the addition of cyclic AMP to disaggregated cells. Although tight aggregation formation is required for initial induction, cyclic AMP is sufficient to maintain expression of pre-spore II genes in disaggregated cells.

Based on the appearance of developmentally regulated enzyme activities, Loomis and co-workers have suggested that *Dictyostelium* development can be interpreted as a series of

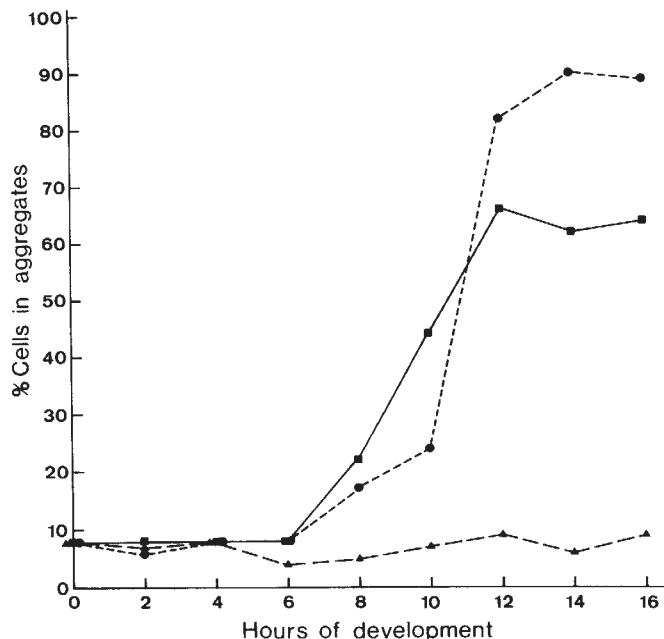


Fig. 3 Developmental kinetics of EDTA-resistant cell aggregate formation. Cells were collected at the indicated times during normal development (squares), starvation in suspension (triangles) or cyclic AMP-pulsed starvation (circles), washed and then resuspended at 5×10^6 cells per ml in PDF containing 10 mM EDTA. This mixture was vortexed until cells were completely dissociated. Aliquots (5 ml) were gently rolled (at 100 r.p.m.) in a roller drum for 30 min after which the percentage of cells in aggregates was determined using a haemocytometer.

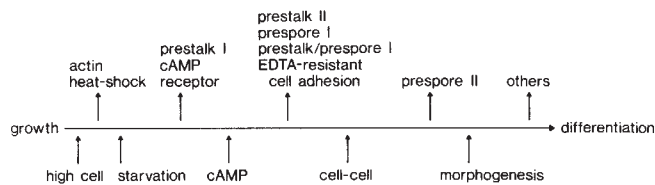


Fig. 4 Schematic diagram of gene expression during *Dictyostelium* development and the factors responsible for induction of specific classes of genes.

'dependent sequences'^{15,16}. When various mutants incapable of completing the developmental programme are ordered based on the enzymatic activities expressed in their 'terminal phenotype', an ordered sequence of gene expression can be defined. Expression of late enzymatic activities is never seen unless the enzymes normally expressed earlier in the developmental programme have been induced. This ordered expression of genes can now be interpreted in light of the extracellular signals that seem to regulate the developmental programme: high cell density, starvation, cyclic AMP and the formation of cellular aggregates.

Received 31 January; accepted 10 April 1984.

- Loomis, W. F. *The Development of Dictyostelium discoideum* (Academic, New York, 1982).
- Gerisch, G. & Hess, B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2118-2122 (1974).
- Gross, J. D., Peacey, M. J. & Trevan, D. J. *J. Cell Sci.* **22**, 645-656 (1976).
- Devreotes, P. N. & Steck, T. L. *J. Cell Biol.* **80**, 300-309 (1979).
- Ochai, H. *et al. Cell Differentiation* **11**, 1-13 (1982).
- Steinman, C. & Parrish, R. W. *Nature* **286**, 621-623 (1980).
- Geltosky, J. E. *et al. Cell* **18**, 391-398 (1979).
- Murray, B. A., Niman, H. L. & Loomis, W. L. *Molec. cell Biol.* **3**, 863-870 (1983).
- Barklis, E. & Lodish, H. F. *Cell* **32**, 1139-1148 (1983).
- Mehdy, M., Ratner, D. & Firtel, R. A. *Cell* **32**, 763-771 (1983).
- Beug, H., Gerisch, G., Kempff, S., Riegel, V. & Cremer, G. *Exp. Cell Res.* **63**, 147-158 (1973).
- Landfear, S. M. & Lodish, H. F. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1044-1048 (1980).
- Chung, S., Landfear, S., Blumberg, D. D., Cohen, N. S. & Lodish, H. F. *Cell* **24**, 785-797 (1981).
- Mangiarotti, G., Ceccarelli, A. & Lodish, H. F. *Nature* **301**, 616-618 (1983).
- Loomis, W. F., White, S. & Dimond, R. *Dev. Biol.* **53**, 171-177 (1976).
- Loomis, W. F., Dimond, R., Free, S. & White, S. in *Eukaryotic Microbes as Model Development Systems* (eds O'Day, D. & Horgen, P.) 177-194 (Dekker, New York, 1978).
- Blumberg, D. D. & Lodish, H. F. *Dev. Biol.* **78**, 285-300 (1980).

Fly and frog homoeo domains show homologies with yeast mating type regulatory proteins

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Homoeotic genes in the bithorax and Antennapedia complexes of *Drosophila melanogaster* appear to specify the developmental fate of segments of the fly. Some of these genes (*Ultrabithorax*, *Antennapedia* and *fushi tarazu*) share homology due to their conservation of a 'homoeo domain'^{1,2} consisting of 60 amino acids. Cross-hybridization and cloning experiments show that the homoeo domain is conserved in a frog (*Xenopus laevis*) gene expressed in early development³ and may also be present in earthworm, beetle, chicken, mouse and human genomes². The extreme conservation found in the amino acid sequences between the *Drosophila* and *Xenopus* domains suggests that the domain has a vital function in the control of early development. Here we report the results of a search made in the Dayhoff sequence bank, which reveals a lesser but apparently significant homology between the homoeo domain and the amino acids coded from parts of the $\alpha 1$ and $\alpha 2$ mating type genes of the yeast *Saccharomyces cerevisiae*^{4,5}.

The degree of homology between the amino acids of the homoeo domains is known to be greater than that between the corresponding DNA sequences (termed homoeo boxes) due to many silent nucleotide changes³. This suggested that the homoeo box is translated into a functional protein, so searches were made in the Dayhoff protein sequence bank, using a consensus sequence from the three *Drosophila* and one *Xenopus* homoeo domains shown in Fig. 1. In the whole bank of 2,372 sequences with a total of 430,262 amino acids, the best homologies were found in the $\alpha 1$ and $\alpha 2$ yeast mating type (*MAT*) proteins towards their carboxyl ends (see Fig. 1).

Although the statistical significance of such homologies does not necessarily imply protein structural similarity (see ref. 6), it

often acts as a guide to a possible relationship. In this case it can be estimated that in a random sequence bank of Dayhoff size there is a relatively small chance of finding such homologies. For example, considering the homology in the 14 amino acids starting at residue 44 in the $\alpha 2$ domain (Fig. 1), 9 agree with the consensus sequence. The chance of finding such homology anywhere in such a bank would be approximately 1 in 10³. Particularly in this region, and from about amino acid 40 (Fig. 1), the homologies are most marked (conservative changes may also be taken into account), but a search with almost the whole length of the consensus sequence also pointed to the yeast genes as having the best homologies to the homoeo domain, with a similarly low probability of this occurring by chance. Perhaps significantly, a large conserved block of amino acids, in all 4 homoeo domains of fly and frog (amino acids 47–55), occurs within the 14 amino acids 44–57, where the homology between the yeast genes and the homoeo domains is best.

It is very striking that the search through the whole sequence bank should have pointed to the *MAT* $\alpha 1$ and $\alpha 2$ genes of yeast, as it has been proposed^{7,8} that these code for proteins which control the expression of many unlinked mating genes and, in a/α diploid cells, possibly of sporulation genes, that is, genes controlling a form of cell differentiation. They could accomplish this by binding of their products to other regulatory loci, since the $\alpha 1$ gene product apparently shares homology with gene-regulatory proteins in prokaryotic systems^{9,10}, particularly in the region of amino acids 32–49 (Fig. 1). A remote but definite functional similarity, possibly some kind of developmental switch acting at the DNA level, is suggested by the present homoeo domain of flies and frogs.

The present search also reveals a mutual homology between the carboxyl ends of the $\alpha 1$ and $\alpha 2$ genes. This homology between the yeast proteins may have functional significance because $\alpha 1$ and $\alpha 2$ are known to act in concert^{7,8} in regulating gene expression in diploid cells. Interestingly, those amino acids shared between $\alpha 1$ and $\alpha 2$ are also the most conserved when compared with the homoeo domain (Fig. 1). This homologous amino acid sequence is also encoded by the gene $\alpha 2$ transcripts, which have the same DNA sequence in this region as $\alpha 2$, and for which a function has not yet been assigned⁵.

At the time we found these homologies, it was believed that

		5										10										15										20									
Fly	Antp	Arg	Lys	Arg	Gly	Arg	Gln	Thr	Tyr	Thr	Arg	Tyr	Gln	Thr	Leu	Glu	Leu	Glu	Lys	Glu	Phe																				
	Ubx	Arg	Arg	Arg	Gly	Arg	Gln	Thr	Tyr	Thr	Arg	Tyr	Gln	Thr	Leu	Glu	Leu	Glu	Lys	Glu	Phe																				
	ftz	Ser	Lys	Arg	Thr	Arg	Gln	Thr	Tyr	Thr	Arg	Tyr	Gln	Thr	Leu	Glu	Leu	Glu	Lys	Glu	Phe																				
Frog	AC1	Arg	Arg	Arg	Gly	Arg	Gln	Ile	Tyr	Ser	Arg	Tyr	Gln	Thr	Leu	Glu	Leu	Glu	Lys	Glu	Phe																				
Yeast	$\alpha 2$	Arg	Gly	His	Arg	Phe	Thr	Lys	Glu	Asn	Val	Arg	Ile	Leu	Glu	Ser	Trp	Phe	Ala	Lys	Asn																				
	$\alpha 1$	Ser	Pro	Lys	Gly	Lys	Ser	Ser	Ile	Ser	Pro	Gln	Ala	Arg	Ala	Phe	Leu	Glu	Gln	Val	Phe																				
		25										30										35										40									
Fly	Antp	His	Phe	Asn	Arg	Tyr	Leu	Thr	Arg	Arg	Arg	Ile	Glu	Ile	Ala	His	Ala	Leu	Cys	Leu																					
	Ubx	His	Thr	Asn	His	Tyr	Leu	Thr	Arg	Arg	Arg	Ile	Glu	Met	Ala	Tyr	Ala	Leu	Cys	Leu																					
	ftz	His	Phe	Asn	Arg	Tyr	Ile	Thr	Arg	Arg	Arg	Ile	Asp	Ile	Ala	Asn	Ala	Leu	Ser	Leu																					
Frog	AC1	His	Phe	Asn	Arg	Tyr	Leu	Thr	Arg	Arg	Arg	Ile	Glu	Ile	Ala	Asn	Ala	Leu	Cys	Leu																					
Yeast	$\alpha 2$	Ile	Glu	Asn	Pro	Tyr	Leu	Asp	Thr	Lys	Gly	Leu	Glu	Asn	Leu	Met	Lys	Asn	Thr	Ser	Leu																				
	$\alpha 1$	Arg	Arg	Lys	Gln	Ser	Leu	Asn	Ser	Lys	Glu	Lys	Glu	Glu	Val	Ala	Lys	Lys	Cys	Gly	Ile																				
		45										50										55										60									
Fly	Antp	Thr	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Trp	Lys	Lys	Glu	Asn																				
	Ubx	Thr	Glu	Arg	Gln	Ile	Glu	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Leu	Lys	Lys	Glu	Ile																				
	ftz	Ser	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Ser	Lys	Lys	Asp	Arg																				
Frog	AC1	Thr	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Trp	Lys	Lys	Glu	Arg																				
Yeast	$\alpha 2$	Ser	Arg	Ile	Gln	Ile	Lys	Asn	Trp	Val	Ser	Asn	Arg	Arg	Arg	Lys	Glu	Lys	Thr	Ile	Thr																				
	unspliced $\alpha 1$	Thr	Pro	Leu	Gln	Val	Arg	Val	Trp	Val	Cys	Asn	Met	Arg	Ile	Lys	Leu	Lys	Tyr	Ile	Leu																				
	spliced $\alpha 1$									Phe	Ile	Asn	Lys	Arg	Met	Arg	Ser	Lys			Stop																				

Fig. 1 Homoeo domains from the fly genes *Antennapedia* (*Antp*), *Ultrabithorax* (*Ubx*) and *fushi tarazu* (*ftz*), and the frog *AC1* gene are aligned with sequences near the carboxyl ends of yeast mating type proteins $\alpha 1$ and $\alpha 2$. The $\alpha 1$ amino acids starting after amino acid 48 are shown both for the spliced and unspliced transcripts. Solid boxes enclose amino acids common between the yeast products and the homoeo domains. Broken line boxes similarly enclose amino acids with conservative changes between the two groups. From ** in $\alpha 2$ there are 19 amino acids to the carboxyl end, whereas at * in $\alpha 1$, there follows only one tyrosine before its end.

the yeast *a1* gene did not contain introns. However, recent work¹¹ has shown that the *a1* gene has two introns, of which the second (52 base pairs long) is inefficiently spliced and in the conserved region. The *a1* amino acid sequences, with this second intron unspliced and spliced, are shown in Fig. 1, and it is seen that in both, carboxyl ends are aligned near the end of the homoeo domain. This splice reveals an interesting feature, for when this intron is removed, the homology in this region is maintained and even increases. Six of the nine amino acids (counting from the splicing site after amino acid 48) are then the same as those found within the homoeo domains, and two others have conservative changes (arginine instead of lysine or vice versa). This is a very unusual event to occur by chance, for the probability of finding most of these homoeo domain amino acids correctly encoded at the unique start position following the splice is less than 1 in 10⁶. One explanation, which could be tested by experiment, might be that both the spliced and the unspliced homoeo-box-homologous transcripts are used to provide alternative DNA binding and control proteins, turning on different sets of genes for mating type and sporulation functions in the *a* and *a/α* type cells. The inefficient splicing already observed may be a first indication of this possibility.

It is, of course, possible that the homologies found might all be chance events, and more information is needed about all three systems—fly, frog and yeast—to prove otherwise. Nevertheless, it is remarkable that the yeast mating type genes should have been found to have homologies with these *Drosophila* homoeotic genes, because both are among the very few genes known to control the stable determination of cell types.

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Note added in proof: The core region of best homology between the *S. cerevisiae* *a1* and *α2* gene products and the homoeo domains has recently been found to be conserved also in a mating-type protein of the fission yeast *Schizosaccharomyces pombe* (M. Smith and D. Beach, personal communication).

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- McGinnis, W., Levine, M. S., Hafen, E., Kuroiwa, A. & Gehring, W. J. *Nature* **308**, 428–433 (1984).
- McGinnis, W., Garber, R. L., Wirz, J., Kuroiwa, A. & Gehring, W. J. *Cell* (in the press).
- Carrasco, A. E., McGinnis, W., Gehring, W. J. & De Robertis, E. M. *Cell* (in the press).
- Astell, C. R. *et al.* *Cell* **27**, 15–23 (1981).
- Tatchell, K., Nasmyth, K. A., Hall, B. J., Astelle, C. & Smith, M. *Cell* **27**, 25–35 (1981).
- Kabsch, W. & Sander, C. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1075–1078 (1984).
- Herskowitz, I. & Oshima, Y. in *The Molecular Biology of the Yeast Saccharomyces* (eds Strathern, J. N., Jones, E. W. & Broach, J. R.) 181–209 (Cold Spring Harbor Laboratory, New York, 1981).
- Nasmyth, K. A. *Rev. Genet.* **16**, 439–500 (1982).
- Matthews, B. W., Ohlendorf, D. H., Anderson, W. F., Fisher, R. G. & Takeda, Y. *Cold Spring Harb. Symp. quant. Biol.* **47**, 427–432 (1982).
- Ohlendorf, D. H., Anderson, W. F. & Matthews, B. W. *J. molec. Evol.* **19**, 109–114 (1983).
- Miller, A. M. *EMBO J.* **3**, 1061–1065 (1984).

Correct transcription of an immunoglobulin κ gene requires an upstream fragment containing conserved sequence elements

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Transcription of the immunoglobulin κ light-chain genes depends on the presence of a TATA box upstream of the leader gene segment^{1,2} and is regulated by an enhancer sequence in the large intron^{3,4}. In studying a rearranged mouse κ light-chain gene we have now found that sequences between –90 and –160 base pairs (bp) upstream of the coding region are essential for correct transcription in gene transfer experiments. This region contains the

deca- and pentadecanucleotide sequences TNATTTGCAT and TGCA^cCTGTGNCCAG, which we call dc and pd, respectively. Sequences related to dc and pd were found upstream of all human and mouse κ -chain variable region (V_{κ}) genes, upstream of λ -chain variable region (V_{λ}) genes, and within the mouse heavy-chain enhancer^{5,6}. An inverted and complementary form of the dc element (ATGCAAATNA, called cd) occurs upstream of all heavy-chain variable region (V_H) genes. The newly defined sequences may be involved in the control of immunoglobulin gene transcription.

We previously reported that a cloned κ gene is not correctly expressed in fibroblasts unless it is placed under the control of a viral promoter⁷. Only incorrectly initiated transcripts are produced from unmodified κ -chain genes⁸. Using the technique of transferring cloned immunoglobulin genes into myeloma cells, it was shown that κ gene expression requires the homologous cell type⁹ and appears to be regulated by an enhancer element in the large intron³. We have now prepared several constructs

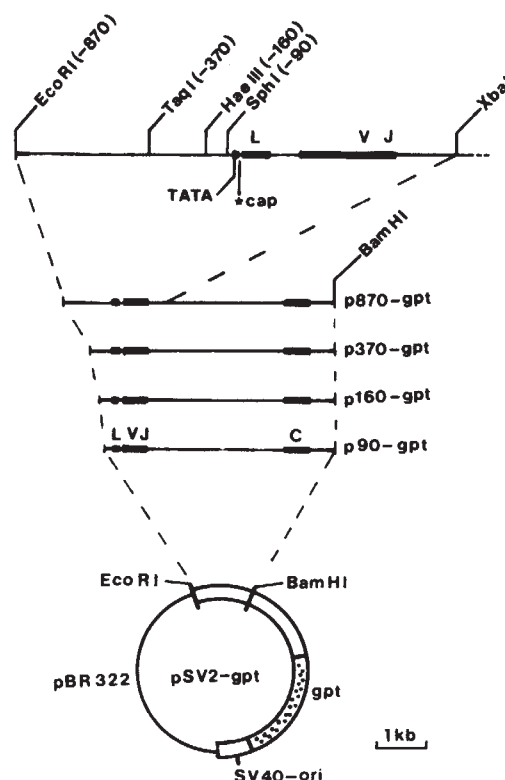


Fig. 1 Construction of κ gene recombinants containing various lengths of the upstream region. The *EcoRI*–*BamHI* fragment containing the immunoglobulin κ gene T1 (ref. 11) with its leader (L), variable (V), joining (J) and constant (C) region segments was shortened at its *TaqI*, *HaeIII* and *SphI* sites, respectively. Those sites were then converted into *EcoRI* sites.

Methods: To construct the plasmid p370-gpt, the plasmid pT1 (ref. 7) was cleaved with *TaqI*, the ends of all fragments were filled in with Klenow polymerase and *EcoRI* linkers were added. After digestion with *EcoRI* and *XbaI* (at the single *XbaI* site of pT1), the *EcoRI*–*XbaI* fragment containing the V region was purified by agarose gel electrophoresis and ligated to the large *EcoRI*–*XbaI* fragment of pT1, resulting in the intermediate plasmid p370. To construct the plasmid p160-gpt, *EcoRI* linkers were added to a *HaeIII* digest of pT1, recut with *EcoRI* and *XbaI*, and the V gene containing the *EcoRI*–*XbaI* fragment was further treated as described for p370-gpt, resulting in the plasmid p160. To construct p90-gpt, the *SphI* fragments of pT1 were made blunt ended with T4 polymerase. *EcoRI* linkers were added and the fragments recut with *EcoRI* and *BamHI*. The shortened *EcoRI*–*BamHI* fragment was ligated with the large *EcoRI*–*BamHI* fragment of pBR322, resulting in plasmid p90. (The *XbaI* cutting step is not necessary here as the κ gene region which has several *TaqI* and *HaeIII* sites contains only one *SphI* site). The κ gene-containing *EcoRI*–*BamHI* fragments of plasmids pT1, p370, p160 and p90 were inserted into the vector pSV2-gpt, resulting in the recombinants p870-gpt, p370-gpt, p160-gpt and p90-gpt.

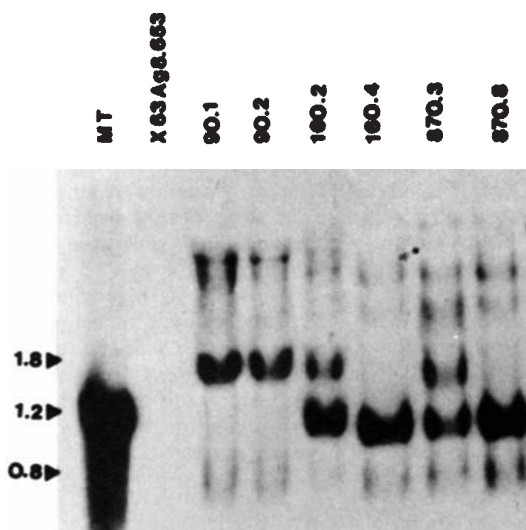


Fig. 2 κ gene transcripts in transformed cell lines. The X63Ag8.653 myeloma line obtained from H. Saumweber (Tübingen) contains one K^- allele (F.G.F., E. Neumann and H.G.Z., manuscript in preparation) and does not produce any κ gene transcripts. For transfection by the electric field method¹³ cell suspensions were exposed to short electric impulses in the presence of high concentrations of dissolved plasmid DNA. The transformants were isolated by gpt selection essentially as described in ref. 10 but with stepwise increases in the concentration of mycophenolic acid from 0.25 to 6 $\mu\text{g ml}^{-1}$ (F.G.F. *et al.*, manuscript in preparation). Fifteen cell lines were established (90.1–90.6, 160.2–160.4 and 870.3–870.8). Northern blot analysis was performed with total cellular RNA as described previously⁷. The hybridization probe was the cloned *HindIII*–*BamHI* fragment of the mouse C_κ region. The 1.2-kb κ mRNA (arrowhead) is present in myeloma T (MT)¹¹ and in the p160-gpt and the p870-gpt transformants; it is absent from the p90-gpt transformants although these cells expressed κ determinants in the screening procedure (see text). The 1.8-kb transcript band initiates in vector sequences (see also Fig. 3). It is absent from cell lines 160.4 and 870.8, probably reflecting the different integration sites of vector and κ gene sequences in the host genome. Additional weak bands in the upper part of the blot are probably splicing intermediates while the weak band at 0.8 kb may be an aberrant splicing product.

composed of different lengths of the region upstream of a κ gene. Plasmids p90-gpt, p160-gpt, p370-gpt and p870-gpt contain, in the vector pSV2-gpt (ref. 10), the mouse κ gene T1 (ref. 11) with 90, 160, 370 and 870 bp, respectively, of its upstream region (Fig. 1). The mouse lymphoid cell line X63 Ag8.653 (ref. 12), which does not produce κ gene transcripts (Fig. 2), was transfected with the two shorter and the longest plasmids, using the electric field method¹³ for transfection; 5–7% of all transformants as selected by the gpt method¹⁰ were positive for κ determinants according to an enzyme-linked immunoassay⁷. Fifteen transformed cell lines were established. Southern blot hybridization with a C_κ region probe revealed (data not shown) that all transformants contained one to five copies of the cloned κ gene integrated into the genome.

κ -specific transcripts of the transformed cell lines were analysed by Northern blot hybridization (examples shown in Fig. 2). The correct 1.2-kilobase (kb) transcript as it occurs in myeloma T (from which the κ gene T1 was originally cloned) was found only in those cell lines which had been transfected with constructs containing 160 bp or more of the upstream region. The absence of the 1.2-kb transcript from all six p90-gpt transformants shows that sequences upstream of position –90 are essential for correct κ gene transcription. As the amounts of the 1.2-kb transcript are, on the average, the same in the p160-gpt and the p870-gpt transformants, sequences upstream of position –160 do not seem to influence transcription in our system.

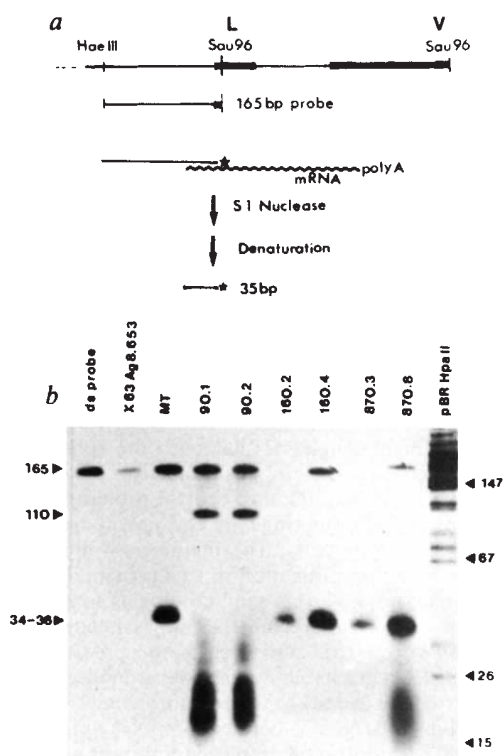


Fig. 3 Determination of initiation sites of κ gene transcripts. In S_1 mapping experiments¹⁴ κ -specific RNAs were detected in total cellular RNA by an end-labelled probe²⁴. *a*, The mapping strategy. The 165-bp *HaeIII*–*Sau96* fragment spanning the upstream region was prepared as a probe. *b*, The S_1 -protected fragments on a 12% polyacrylamide-urea gel. The cap site of the κ gene T1 is 23–25 bp upstream of the ATG codon²⁵, resulting in a protected band of 34–36 bp in the myeloma T control and the p160-gpt and p870-gpt transformants. RNA from myeloma X63 Ag8.653 as a negative control and the p90-gpt transformants do not show this band. In the latter transformants a 110-bp band is present, which indicates protection of the probe up to the *SphI* site at position –90 where the homology between the probe and the transcript ends. This does not reflect a cap site but is due to the construction of the p90-gpt plasmids (Fig. 1); it indicates that the transcript starts upstream of position –90 in the vector part of the recombinant plasmid (see Fig. 2 legend). For clones 160.2 and 870.3, the vector-derived 1.8-kb band gives rise to protection of the probe in its full length. The corresponding band (165 nucleotides) is weak; while it cannot be seen in print, it is clearly visible in the original autoradiograph. The smear at about 20 nucleotides seen in the lanes of clones 90.1, 90.2 and 870.8 is due to incomplete digestion with S_1 nuclease.

S_1 mapping experiments¹⁴ were performed to confirm that the transformants initiate transcription correctly (Fig. 3). The 34–36-nucleotide long fragments expected for correctly initiated transcripts were produced from the RNA of the p160-gpt and p870-gpt transformants as well as from myeloma T RNA, but were not seen in experiments with the RNAs from p90-gpt transformants or control cells. S_1 protection experiments with a probe spanning the small intron demonstrated that κ mRNAs of the p90-gpt, p160-gpt and p870-gpt transformants are correctly spliced (data not shown). According to fluorographic experiments (as in ref. 7), the latter two cell lines also produce κ proteins at a similar level to the mouse lymphoid cell line NS1, which produces but does not secrete a κ protein (ref. 15; data not shown).

The apparent importance of DNA sequences upstream of position –90 for the correct transcription of the κ gene T1 prompted us to search in this region for sequence elements which are conserved among V_κ genes and possibly other immunoglobulin genes and which might be functionally important. Apparently, the TATA box 51 bp upstream of the κ gene T1 and the enhancer sequence in the large intron are not

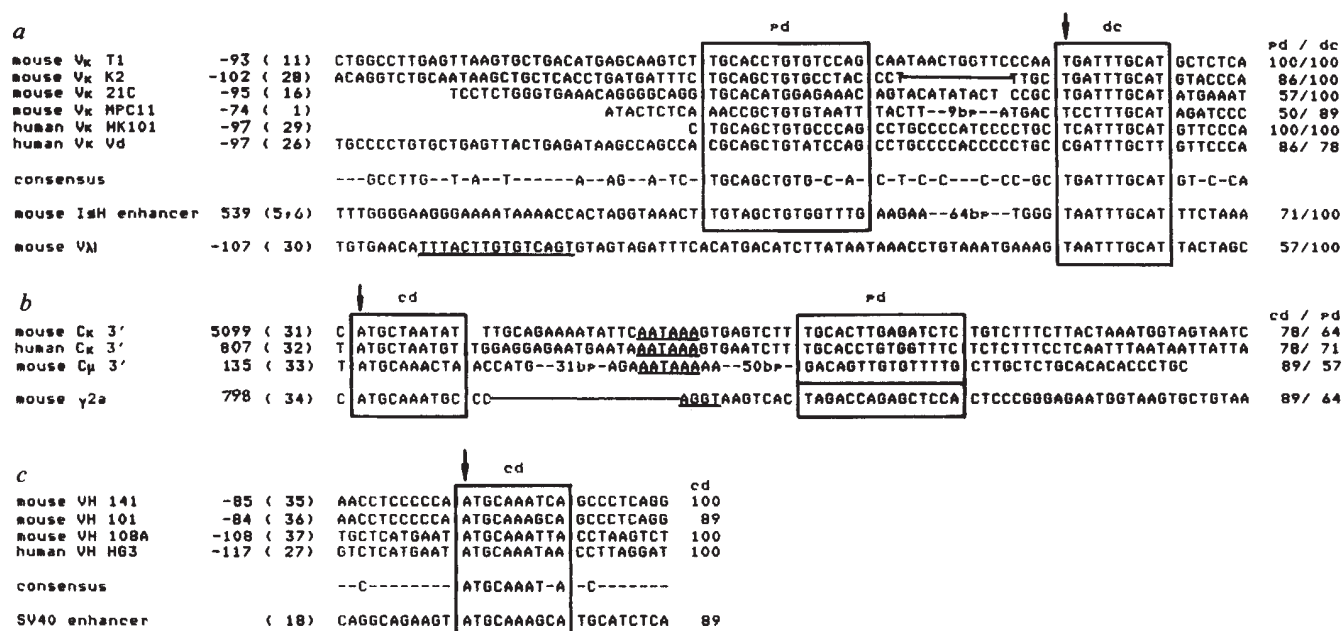


Fig. 4 The dc, cd and pd related elements in immunoglobulin gene and other gene regions. The sequence elements are boxed. The numbers on the left side refer to the position of the first nucleotides of the elements (vertical arrows); for the V_{κ} , V_{λ} and V_H genes the numbers give the distance to the start of the coding region; in the other sequences they are the ones used in the respective publications (references in parentheses). The numbers on the right-hand side are homologies (per cent) between the elements in the particular sequence and the dc, pd and cd consensus sequences, respectively. The sequences were aligned with the help of the DNMAHO program; regions homologous to the dc, cd and pd elements were searched for by the DNSEPA program (for the programs written by P. S. Neumaier see ref. 26). In the lines labelled 'consensus', nucleotides show up which occur in more than 50% of the sequences compared. **a**, dc and pd related elements upstream of V_{κ} and V_{λ} gene segments. Sequences are presented which show the most divergence. Sequences which are very similar to those presented (mouse V_{κ} , V_{λ} , human V_{κ}) are not shown. The consensus sequences of the dc and pd element were defined on the basis of all known mouse V_{κ} and human V_{κ} genes. A corrected version (R. P. Perry, personal communication) of the MPC11 sequence of ref. 1 is shown. The published sequence of the gene for the MPC11 light-chain fragment³⁸, which has an unusually short distance between the dc element and the ATG codon, is not included in the comparison; instead the V_{κ} 21 sequence¹⁶ is shown from which the former one was supposedly derived³⁸. dc and pd related elements are also found upstream of other V genes, for example, the human V_{κ} II, V_{κ} III and V_{κ} IV genes, which contain the elements in similar relative positions but within different surrounding sequences (H.-G. Klobeck, A. Meindl and M. Pech, unpublished). In these subgroups the pd elements are more variable but still show significant homologies to the pd consensus sequence; the dc elements are highly homologous within all human V_{κ} genes. Pseudogenes, some of which have altered dc and pd elements, were not scored. Part of the sequence of the IgH enhancer is included in the comparison. A pd related element in the V_{λ} gene is underlined. **b**, The cleavage polyadenylation recognition signals (underlined) of two κ genes and one μ gene (μ_s) are flanked by cd and pd related elements. The splice site of the mouse γ 2a hinge exon (underlined) is flanked by a cd element and by an element which is homologous to the inverted and complementary version of pd. **c**, cd related elements upstream of V_H genes and in the 72-bp repeat of SV40. Examples of V_H gene sequences are also shown. The cd related elements which were found upstream of all sequenced V_H genes lie in some cases within a conserved segment which is discussed as part of a hypothetical stem-loop structure²⁷. A computer search for dc and cd elements at a 89% homology level showed that they were not present in pBR322 (ref. 39), BKV virus⁴⁰ or the human insulin gene region⁴¹ but they were found in the 5' flanking region of the chicken ovalbumin gene⁴² and in the sea urchin histone gene cluster⁴³ (preferentially in noncoding regions); dc was also found in the dispersed repetitive R sequences⁴⁴. pd (and enhancer core) related elements were found in the neighbourhood of dc elements in, for example, the human β - and ϵ -globin gene regions^{45,46}. dc and pd related elements also occur upstream of the genes for mouse and human class II histocompatibility antigens⁴⁷⁻⁵⁰, another group of genes expressed in lymphocytes.

sufficient to direct correct transcription. The CCAAT sequence at position -97 is deleted in the non-functional plasmid p90-gpt, but because this sequence is found only in some V_{κ} gene regions it does not seem to be a general regulatory element. However, sequences homologous to the decanucleotide 5'-TNATTTGCAT-3' (dc element) and to the pentadecanucleotide 5'-TGCACTGTGNCCAG-3' (pd element) of the T1 gene are found upstream of all human and mouse V_{κ} genes which have been sequenced and also upstream of the two mouse V_{λ} genes (Fig. 4a). The dc and pd elements are found in the other V_{κ} gene regions at similar positions to those in the T1 gene (for the V_{κ} 21C sequence¹⁶ see Fig. 4 legend). The pd-related elements are generally less well conserved than the dc elements (Fig. 4a). The inverted and complementary version of the dc element (5'-ATGCAAAATNA-3'), which we call cd element, is found in the -100-bp region of all V_H genes. The dc, pd and cd elements are homologous to parts of the IgH and simian virus 40 (SV40) enhancer sequences^{5,6,17,18} (Fig. 4a, c). This homology may have a functional significance; it may also reflect a common evolutionary origin of regulatory elements.

The dc, cd and pd elements are not unique to the upstream regions of the immunoglobulin genes. Related sequences are

found, for instance, near the cleavage polyadenylation recognition signals of two immunoglobulin genes (Fig. 4b) and in the neighbourhood of some other genes (Fig. 4 legend). While the functional significance of these scattered sequence elements is unclear, it is reasonable to assume that the elements upstream of the V genes are involved in the regulation of transcription. They are conserved in sequence and in their distance to the coding region. A role in transcription is also suggested by our deletion analysis and by the observation that the elements are frequently located within DNase I-hypersensitive sites which, in turn, play a part in gene activity (see ref. 19 for review). Such DNase I sites are found upstream of rearranged mouse²⁰ and human κ genes (V. Pospelov, H.-G. Klobeck and H.G.Z., manuscript in preparation). They have also been assigned to other regions which contain the sequence elements (Fig. 4): upstream of a V_H gene²¹ (tentatively), in the heavy chain enhancer region²¹, and in the neighbourhood of the cleavage polyadenylation recognition signal of a human κ gene (V. Pospelov, H.-G. Klobeck and H.G.Z., in preparation). It may be speculated that the dc and pd elements upstream of the V_{κ} genes are involved in tissue-specific initiation of transcription and that the dc and cd elements are involved in the coordination of light- and

heavy-chain expression²². Note, in this context, that short nucleotide sequences have been implicated in the coordinate induction of genes²³. Further work is required to elucidate in detail the role of dc, cd and pd elements in immunoglobulin gene transcription.

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1. Kelley, D. E., Coleclough, C. & Perry, R. P. *Cell* **29**, 681–689 (1982).
2. Bentley, D. L., Farrell, P. J. & Rabbitts, T. H. *Nucleic Acids Res.* **10**, 1841–1856 (1982).
3. Picard, D. & Schaffner, W. *Nature* **307**, 80–82 (1984).
4. Queen, C. & Baltimore, D. *Cell* **33**, 741–748 (1983).
5. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729–740 (1983).
6. Gillies, S. D., Morrison, S. L., Oi, V. & Tonegawa, S. *Cell* **33**, 717–728 (1983).
7. Falkner, F. G. & Zachau, H. G. *Nature* **298**, 286–288 (1982).
8. Gillies, S. D. & Tonegawa, S. *Nucleic Acids Res.* **11**, 7981–7997 (1983).
9. Stafford, J. & Queen, C. *Nature* **306**, 77–79 (1983).
10. Mulligan, R. C. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2072–2076 (1981).
11. Altenburger, W., Steinmetz, M. & Zachau, H. G. *Nature* **287**, 603–607 (1980).
12. Kearney, J. F., Radbruch, A., Liesegang, B. & Rajewsky, K. *J. Immun.* **123**, 1548–1550 (1979).
13. Neumann, E., Schaefer-Ridder, M., Wang, Y. & Hofschneider, P. H. *EMBO J.* **1**, 841–845 (1982).
14. Berk, A. J. & Sharp, P. A. *Cell* **12**, 721–732 (1977).
15. Köhler, G., Howe, S. C. & Milstein, C. *Eur. J. Immun.* **6**, 292–295 (1976).
16. Heinrich, G., Traunecker, A. & Tonegawa, S. *J. exp. Med.* **159**, 417–435 (1984).
17. Benoist, C. & Chambon, P. *Nature* **290**, 304–310 (1981).
18. Gruss, P., Dhar, R. & Khoury, G. *Proc. natn. Acad. Sci. U.S.A.* **78**, 943–947 (1981).
19. Igo-Kemenes, T., Hörz, W. & Zachau, H. G. *A. Rev. Biochem.* **51**, 89–121 (1982).
20. Weischet, W. O., Glotov, B., Schnell, H. & Zachau, H. G. *Nucleic Acids Res.* **10**, 3627–3645 (1982).
21. Mills, F. C., Fisher, M. L., Kuroda, R., Ford, A. M. & Gould, J. H. *Nature* **306**, 809–812 (1983).
22. Falkner, F. G. thesis, Univ. München (1984).
23. Davidson, E. H., Jacobs, H. T. & Britten, R. J. *Nature* **301**, 468–470 (1983).
24. Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **7**, 1175–1193 (1979).
25. Bodary, S. & Mach, B. *EMBO J.* **1**, 719–724 (1982).

transfer experiments by the electric field method, P. S. Neumaier and B. Straubinger for help in the computing, and R. P. Perry for information on the κ gene sequences of MPC11 and the MPC11 light-chain fragment. The work was supported by Bundesministerium für Forschung und Technologie and Fonds der Chemischen Industrie.

26. Pech, M. *et al. J. molec. Biol.* (in the press).
27. Rechavi, G., Ram, D., Glazer, L., Zakut, R. & Givol, D. *Proc. natn. Acad. Sci. U.S.A.* **80**, 855–859 (1983).
28. Nishioka, Y. & Leder, P. *J. biol. Chem.* **255**, 3691–3694 (1980).
29. Bentley, D. L. & Rabbitts, T. H. *Cell* **32**, 181–189 (1983).
30. Hozumi, N. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 7019–7023 (1981).
31. Max, E. E., Maizel, J. V. & Leder, P. *J. biol. Chem.* **256**, 5116–5120 (1981).
32. Hieter, P. A., Max, E. E., Seidman, J. G., Maizel, J. V. & Leder, P. *Cell* **22**, 197–207 (1980).
33. Early, P. *et al. Cell* **20**, 313–319 (1980).
34. Olo, R., Auffray, C., Morchamps, C. & Rougeon, F. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2442–2446 (1981).
35. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. *Nature* **286**, 676–683 (1980).
36. Kataoka, T., Nikaio, T., Miyata, T., Moriwaki, K. & Honjo, T. *J. biol. Chem.* **257**, 277–285 (1982).
37. Givol, D. *et al. Nature* **292**, 426–430 (1981).
38. Seidman, J. G. & Leder, P. *Nature* **286**, 779–783 (1980).
39. Sutcliffe, J. G. *Cold Spring Harb. Symp. quant. Biol.* **43**, 77–90 (1979).
40. Seif, I., Khoury, G. & Dhar, R. *Cell* **18**, 963–977 (1979).
41. Ullrich, A., Dull, T. J., Gray, A., Brosius, J. & Sures, I. *Science* **209**, 612–615 (1980).
42. Robertson, M. A. *et al. Nature* **278**, 370–372 (1979).
43. Birnstiel, M. *et al. Proc. Alfred Benzon Symp.* **13**, 117–132 (1979).
44. Gebhard, W. & Zachau, H. G. *J. molec. Biol.* **170**, 255–270 (1983).
45. Lawn, R. M., Efstratiadis, A., O'Connell, C. & Maniatis, T. *Cell* **21**, 647–651 (1980).
46. Baralle, F. E., Shoulders, C. C. & Proudfoot, N. J. *Cell* **21**, 621–626 (1980).
47. Hyldig-Nielsen J. J. *et al. Nucleic Acids Res.* **11**, 5055–5071 (1983).
48. Mathis, D. J., Benoist, C. O., Williams, V. E. II, Kanter, M. R. & McDevitt, H. O. *Cell* **32**, 745–754 (1983).
49. Malissen, M., Hunkapiller, T. & Hood, L. *Science* **221**, 750–754 (1983).
50. Das, H. K., Lawrence, S. K. & Weissman, S. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3543–3547 (1983).

New photoactivatable cyclic nucleotides produce intracellular jumps in cyclic AMP and cyclic GMP concentrations

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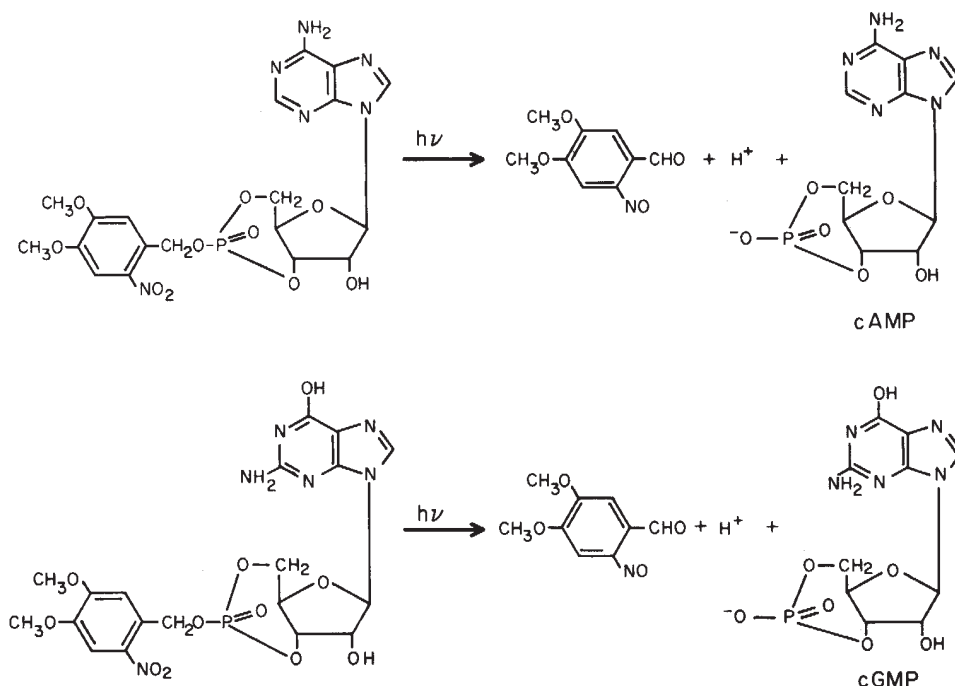
The cyclic nucleotides cyclic AMP and cyclic GMP are important intracellular messengers mediating the responses to neurotransmitters and neurohormones and regulating cellular function over a wide range of time scales^{1,2}. Despite the widespread acceptance of this second messenger mechanism^{3,4} in many systems, much remains unknown about their mechanism of action, except that such events are associated with increases or decreases in intracellular cyclic nucleotides. Quantitative descriptions of cyclic nucleotide-dependent processes are hampered by the absence of a means by which intracellular cyclic nucleotide concentrations can be accurately controlled. We have now designed, synthesized and characterized new, substituted⁵ photolabile cyclic nucleotide analogues, the 4,5-dimethoxy-2-nitrobenzyl esters of cyclic AMP and cyclic GMP (Fig. 1), which are physiologically inert before irradiation and which liberate free cyclic AMP or cyclic GMP on absorption of a photon. The thermal properties and photolysis rates and efficiencies of light-induced release of cyclic nucleotides from these analogues are more favourable than for the simple *o*-nitrobenzyl derivatives used previously⁶. These molecules should permit intracellular 'concentration jumps' of cyclic AMP or cyclic GMP to be produced in cells under physiological investigation with spatial and temporal resolution unmatched by conventional techniques.

The absorption spectra of the 4,5-dimethoxy-2-nitrobenzyl esters of cyclic AMP and cyclic GMP are significantly redshifted

from the unsubstituted *o*-nitrobenzyl esters^{6,7}, displaying absorption maxima at 350 nm (Fig. 2). Irradiation of the esters in solution at wavelengths of 280–420 nm results in liberation of free cyclic AMP or cyclic GMP with no detectable side reactions. The reaction efficiency is independent of ester concentration and is constant in solutions containing up to 5% dimethyl sulphoxide (DMSO). The reaction efficiency is similarly not influenced by the ionic strength, pH or dielectric constant of the medium. In our optical system (xenon short-arc flash lamp, ~200 J discharge energy, ~1 ms flash duration^{8,9}), using a 300-nm cutoff filter (Schott WG 295), ~5% of the molecules are converted per flash. This conversion efficiency is constant when irradiation is effected with light of wavelengths between 300 and 360 nm (using various cutoff filters) and falls off sharply at wavelengths >400 nm. The efficiency of conversion can be improved by a factor of three when a 280-nm cutoff filter is substituted for the longer wavelength filters used here. An additional factor of two is gained when the 75-mm secondary focusing lens is replaced by a 50-mm lens. It is also technically possible to produce higher per-flash conversions by using a laser^{10,11} or by increasing the number and/or size of the storage capacitors in the flash lamp power supply^{6,8,9}. Flash photolysis studies indicate that after a single flash, cyclic nucleotides are liberated from the dimethoxy analogues in less than 5 ms (J.M.N. and H.A.L., unpublished), the present limit of resolution of the flash photolysis apparatus. This rate is ~200 times faster than the rate of photorelease from the unsubstituted *o*-nitrobenzyl ester derivatives (J.M.N. and H.A.L., unpublished). In contrast to the results obtained with *o*-nitrobenzyl cyclic GMP⁶, the efficiency of photorelease from 4,5-dimethoxy-2-nitrobenzyl cyclic GMP is equivalent to that from the cyclic AMP derivative. Although it is unclear why *o*-nitrobenzyl cyclic GMP photolysis proceeds with half the efficiency of the cyclic AMP analogue⁶, preliminary results suggest that competing, secondary side reactions reduce the yield. Regardless of the correct explanation, we can conclude that the photolysis of 4,5-dimethoxy-2-nitrobenzyl cyclic GMP proceeds free of complications.

In the absence of light, 4,5-dimethoxy-2-nitrobenzyl cyclic AMP causes no measurable changes in the waveform or duration of the action potential or the slow inward Ca²⁺ current (*I_{si}*) in isolated atrial trabeculae from frog heart (*Rana esculenta*) at

Fig. 1 Irradiation of the 4,5-dimethoxy-2-nitrobenzyl esters of cyclic AMP and cyclic GMP yields 4,5-dimethoxy-2-nitrosobenzaldehyde, a proton and the ionized acids of cyclic AMP or cyclic GMP. The esters were synthesized by modified procedures of those described previously^{7,22}. Briefly, 4,5-dimethoxy-2-nitrobenzaldehyde, freshly prepared and purified²³, was converted to the tosylhydrazone derivative by refluxing with *p*-toluenesulphonylhydrazide in methanol (MeOH) for 6 h²⁴. The tosylhydrazone which precipitates from the reaction mixture was decomposed to 4,5-dimethoxy-2-nitrophenyldiazomethane by stirring in aqueous 10% NaOH for 12–16 h at room temperature²⁵. The crystalline diazomethane derivative which forms is isolated by filtration and is stable at 0 °C for several weeks. Decomposition of this compound is quite rapid at room temperature ($t_{1/2}$ 2–3 days). The diazomethane was coupled directly to the free acid of cyclic AMP or cyclic GMP by stirring a concentrated solution of the reactants in DMSO for 1–2 days.



The disappearance of the characteristic red colour of the diazo compound signalled completion of the reaction; alternatively, the course of the reaction can be monitored by TLC on silica gel ($CHCl_3/MeOH = 5:1$, v/v). The newly synthesized esters were separated from the reaction mixture by preparative TLC (eluted from the silica with MeOH), recovered quantitatively following solvent evaporation and recrystallized from MeOH/ H_2O mixtures or lyophilized dioxane/ H_2O . For both derivatives, the dimethoxynitrobenzyl esters crystallized as pale yellow needles (m.p. 150–160 °C(d) and 160–180 °C(d) for the cyclic AMP and cyclic GMP analogues, respectively). The 1H -NMR (90 MHz) spectra (DMSO- d_6) revealed resonances (in p.p.m.) at 7.8 (s, 1H), 7.4 (s, 1H), 5.5 (d, 3Hz, 2H) and 3.9 (s, 6H) which could be assigned to the *o*-nitrobenzyl moiety. As expected⁷, the *o*-nitrobenzyl esters synthesized by this method are obtained as diastereomeric mixtures. ^{31}P -NMR spectra (DMSO- d_6) permitted isomer assignments at -5.1 (axial) and -3.6 (equatorial) and determinations of isomeric product ratios: the 4,5-dimethoxy-2-nitrobenzyl esters of cyclic AMP and cyclic GMP are both obtained as the axial and equatorial isomers in a 60(axial):40(equatorial) ratio. HPLC analyses (C_{18} column: 30% acetonitrile in H_2O) revealed <2% contamination of the esters by the free acids of cyclic AMP and cyclic GMP and permitted separation of the axial ($R_F = 0.65$) and equatorial ($R_F = 0.52$) isomers. The 4,5-dimethoxy-2-nitrobenzyl esters of cyclic AMP and cyclic GMP are only partially soluble in water but, if dissolved first in DMSO, then diluted into the appropriate Ringer's solution, concentrations of several hundred micromolar can be achieved (DMSO concentrations never exceed 1% in our chemical or physiological experiments). Although the esters are indefinitely stable in crystalline form at room temperature in the absence of light, in aqueous solutions they slowly undergo thermal hydrolysis to liberate free cyclic AMP or cyclic GMP; the $t_{1/2}$ values are 36 h and 100 h for the axial and equatorial isomers, respectively, at room temperature (as assayed by HPLC). Frozen solutions of both compounds appear to be stable for at least 30 days.

concentrations up to 200 μM . Higher concentrations were not tested, as maximal responses ($\sim$ threefold increases in I_{si} amplitude) were produced by single flashes in the presence of 100 μM of this compound. In the concentration range 2–100 μM , flashes produce large increases in the amplitude of I_{si} with no measurable changes in the kinetics or the voltage dependence of the current (Fig. 3). The absolute magnitude of the increase in I_{si} , the time to the peak response and the recovery time all depend on the amplitude of the concentration jump of cyclic AMP produced (Fig. 4), as shown previously for *o*-nitrobenzyl cyclic AMP⁶. The amplitude of the jump can be controlled by varying the light intensity, the wavelength of illumination or the starting concentration of the ester. Presumably, the time course reflects the biochemical events (that is, interaction between cyclic AMP and cyclic AMP-dependent protein kinase(s), kinase dissociation, protein phosphorylation/dephosphorylation and cyclic AMP hydrolysis) which are thought to be involved in the regulation of I_{si} by cyclic AMP^{12–14}. The wavelength dependence of the increase in I_{si} amplitude agrees in several respects with that expected from the solution chemistry of this compound: (1) light of wavelengths 300–360 nm is equally effective in increasing I_{si} amplitude; (2) the response is attenuated over 360–400 nm and vanishes above 430 nm; and (3) at 400 nm the response is $\sim 5\%$ of the value at 350 nm. Following single flashes, small (~ 2 –5%), but reproducible, increases in I_{si} amplitude are observed within 150–200 ms, in agreement with our previous findings⁶. We have not observed increases in I_{si} at shorter times in these experiments even though the nonspecific flash

artefacts^{6,9} seem to be eliminated and these molecules allow larger and more rapid concentration jumps than the *o*-nitrobenzyl analogues used previously⁶. In similar experiments in the presence of the photosensitive calcium channel antagonist nifedipine^{15,16}, we find that flash effects are observed within 1 ms and are completed within 10 ms¹⁷. The relatively slow time course of the effects following cyclic AMP jumps, therefore, seems to reflect the biochemical events thought to underlie the modulation of I_{si} by cyclic AMP^{12–14,18–20}.

The delayed rectifier (K^+) current in these cells is increased by cyclic AMP concentration jumps; however, the concentration dependence and the kinetics of this effect do not seem to parallel that seen in the modulation of I_{si} (refs 6, 21). The background current and the muscarinic K^+ conductance, on the other hand, seem unaffected by cyclic AMP jumps. We find no effects of flashes in the presence of 4,5-dimethoxy-2-nitrobenzyl cyclic GMP at concentrations up to 500 μM , nor are the effects of cyclic AMP jumps attenuated or augmented when both 4,5-dimethoxy-2-nitrobenzyl cyclic AMP and cyclic GMP are present in the perfusion solution. These findings support our previous conclusions⁶ that cyclic GMP does not modulate the slow inward current in frog heart¹⁸.

We have made no direct measurements of the amount of cyclic AMP or cyclic GMP actually produced intracellularly following flashes. Because the esters probably equilibrate rapidly and the reaction efficiency is relatively insensitive to environment, it is likely that the intracellular concentration jumps are at least as large as those produced extracellularly. If so, single

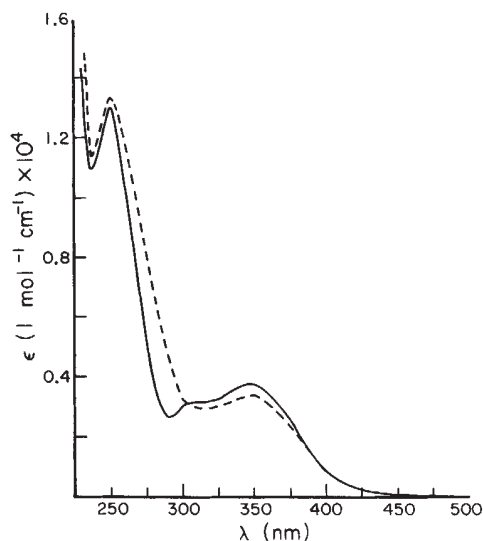


Fig. 2 Absorption spectra of the 4,5-dimethoxy-2-nitrobenzyl esters of cyclic AMP (—) and cyclic GMP (---) in 0.2% DMSO in water at 100 μ M. Following irradiation, the spectra shift only slightly (data not shown), due to the combined absorbances of the cyclic nucleotide and the nitrosobenzaldehyde photoproducts. The axial and equatorial isomers of 4,5-dimethoxy-2-nitrobenzyl cyclic AMP and cyclic GMP display identical absorption spectra and, in addition, react with equal efficiencies and rates to yield free cyclic AMP or cyclic GMP on absorption of a photon. In all physiological experiments, therefore, nitrobenzyl esters obtained as diastereomeric mixtures were used.

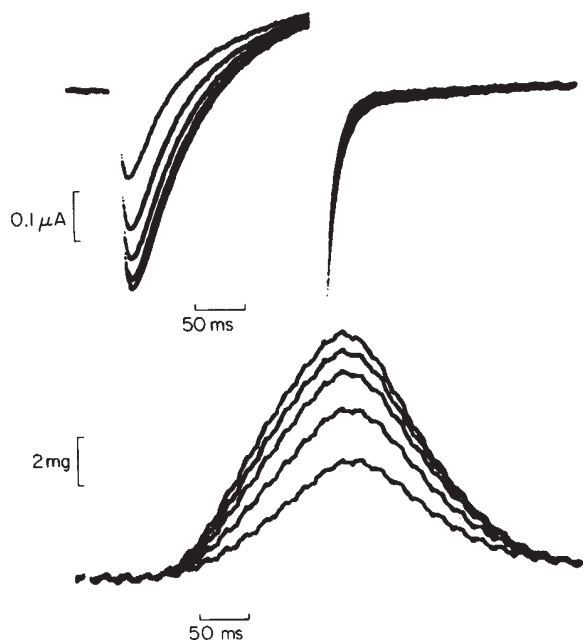


Fig. 3 Waveforms of I_{si} and twitch tension in an isolated atrial trabecula in double sucrose-gap voltage-clamp conditions. The Ringer's solution contained 1 μ M tetrodotoxin to suppress the fast, inward Na^+ current and 50 μ M 4,5-dimethoxy-2-nitrobenzyl cyclic AMP. The voltage was held at the resting potential and depolarized by 80 mV at 5-s intervals. The steady-state level of I_{si} is smallest in the first sweep. One second before the second sweep a single flash was delivered, resulting in a large increase in the amplitude of I_{si} . The current continued to increase over the next several sweeps, levelled off and finally returned to the pre-flash value (recovery not shown). Tension amplitude increased following the flash with a time course and dose dependence²⁶ which closely parallel the increase in I_{si} .

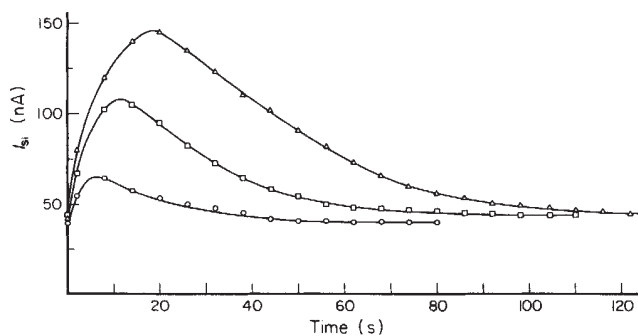


Fig. 4 Time course of the increase and recovery in I_{si} amplitude following a single light flash in preparations bathed in 10 μ M (○), 25 μ M (□) and 100 μ M (△) 4,5-dimethoxy-2-nitrobenzyl cyclic AMP. The flash was delivered at time zero and the voltage stepped every 6 s. Other conditions were the same as for the experiment of Fig. 3. The absolute magnitude of the flash-induced increase in I_{si} is variable among preparations and dependent on the steady-state amplitude of the current.

flashes here yield 0.1–5 μ M cyclic AMP from starting ester concentrations of 2–100 μ M. As preferential partitioning into membranes could produce non-uniform jumps, this question might better be approached with membrane-impermeant analogues. We anticipate that light-activated cyclic nucleotides will be useful for the production of intracellular concentration jumps of cyclic AMP and cyclic GMP in virtually any cell or tissue which is reasonably transparent and lacks a physiological response to light. In experiments like those described here, this technique can produce concentration steps in the range of 0.1–10 μ M following single light flashes. The absolute magnitude of the intracellular jumps possible in other cell types would depend on the stabilities and enzymatic activities of these compounds in the absence of light, the details of their intracellular distributions, and the optical properties of the preparation.

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- Kupferman, I. A. *Rev. Neurosci.* **2**, 447–465 (1979).
- Drummond, G. I. *Adv. Cyclic Nucleotide Res.* **15**, 373–494 (1983).
- Sutherland, E. W. & Rall, T. W. *Pharmac. Rev.* **12**, 265–299 (1960).
- Sutherland, E. W. & Robison, G. A. *Pharmac. Rev.* **18**, 145–161 (1966).
- Patchornik, A., Amit, B. & Woodward, R. B. *J. Am. chem. Soc.* **92**, 6333–6335 (1970).
- Nargeot, J., Nerbonne, J. M., Engels, J. & Lester, H. A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2395–2399 (1983).
- Engels, J. & Schlaeger, E. J. *J. med. Chem.* **20**, 907–911 (1977).
- Lester, H. A. & Nerbonne, J. M. A. *Rev. Biophys. Bioengng* **11**, 151–175 (1982).
- Nargeot, J. *et al. J. gen. Physiol.* **79**, 657–678 (1982).
- Kaplan, J. H., Forbush, B. & Hoffmann, J. F. *Biochemistry* **17**, 1929–1935 (1978).
- McCray, J. A., Herbette, L., Kihara, T. & Trentham, D. R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7327–7341 (1980).
- Reuter, H. A. *Rev. Physiol.* **41**, 413–424 (1979).
- Reuter, H. A. *Nature* **301**, 569–574 (1983).
- Tsien, R. W. A. *Rev. Physiol.* **45**, 341–358 (1983).
- Schlossmann, V. K. *Arzneimittel-Forsch.* **22**, 60–62 (1972).
- Morad, M., Goldman, T. E. & Trentham, D. R. *Nature* **304**, 635–638 (1983).
- Nerbonne, J. M., Richard, S. & Nargeot, J. (in preparation).
- Trautwein, W., Taniguchi, J. & Noma, A. *Pflügers Arch. ges. Physiol.* **392**, 307–314 (1982).
- Cachelin, A. B., dePeyer, J. E., Kokubun, S. & Reuter, H. *Nature* **304**, 462–464 (1983).
- Bean, B. P., Nowicky, M. C. & Tsien, R. W. *Nature* **307**, 371–375 (1984).
- Garnier, D., Lester, H. A., Nargeot, J., Nerbonne, J. M. & Richard, S. *J. Physiol., Lond.* **344**, 38P (1983).
- Engels, J. & Reids, R. *Experientia* **34**, 14–15 (1978).
- Fetscher, C. A. *Org. Syn. Coll. A*, 735–737 (1963).
- Davies, H. W. & Schwarz, M. J. *org. Chem.* **30**, 1242–1244 (1965).
- Ried, W. & Ritz, M. *Justus Liebigs Annln Chem.* **691**, 50–54 (1966).
- Richard, S., Nerbonne, J. M., Nargeot, J. & Garnier, D. *Pflügers Arch. ges. Physiol.* (submitted).

Science and the State

Eric Ashby

Empire of Knowledge: The Academy of Sciences of the USSR (1917-1970).

By Alexander Vucinich.

University of California Press: 1984. Pp.484. \$29.95, £23.95.

In August 1940 the distinguished Russian geneticist, Academician N.I. Vavilov, was arrested while on a trip to the Ukraine. He was tried on charges of cosmopolitanism and of sabotaging Soviet agriculture, and sentenced to death. The sentence was commuted to life imprisonment and Vavilov died in prison in Saratov in 1943, of pneumonia according to the police record. The Academy of Sciences did nothing to defend him. On the contrary, when the president welcomed T.D. Lysenko into full membership in 1939 he spoke with enthusiasm about Lysenko's campaign to make Western genetics an illegal discipline in the Soviet Union. The editorial board of the *Great Soviet Encyclopedia*, published by the Academy, were instructed to omit Vavilov's name from the second edition: he was expunged from Soviet history.

Less than 20 years later the Academy rehabilitated Vavilov as thoroughly as they had expunged him. They

republished his collective works, sponsored numerous symposia in his honor, issued a volume of glowing reminiscences by friends and admirers . . . initiated prizes honoring his name, and made him one of the most popular subjects of biographical studies [p.320 of *Empire of Knowledge*].

The Academy that turned this somersault in the 1960s is now indeed what Alexander Vucinich calls it: an "empire of knowledge". Its praesidium is responsible for scores of laboratories from Moscow to Siberia and Uzbekistan; it is the matriarch of academies in most of the fifteen republics; its output of publications is immense. Under the words *Akademi Nauk*, its activities cover much more than science and technology: the social sciences, history, philosophy, linguistics. It has, at one time or another, assumed some of the functions performed in Britain by the Royal Society, the Research Councils and the University Grants Committee.

How is it that this elite coterie of scholars was able in 1949 to celebrate Stalin's birthday by telling him, in a telegram, that every science reflected his guiding ideas and creative genius; and, only eight years later, provide the scientific skills to put Sputnik into orbit? The answers to these questions reveal a saga of patient heroism against awful odds. Beneath the servile assertions that in future science would serve ideology, there was a steadfast loyalty to the autonomy of science and the integrity of scholarship. The wheel has turned: today in the Soviet Union, ideology has to serve science. Persecutions continue, but it is in-

dividuals who are harrassed, not scientific theories.

The history of the Academy of Sciences is a drama: the struggle between those who rule and those who think. It is in miniature the history of the Russian people. In his

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Hero — N.I. Vavilov (1887-1943)...

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...and villain — T. D. Lysenko (1898-1976).

masterly study of the Academy Vucinich writes coolly, sometimes with savage irony, about the episodes of tragedy and triumph which have brought the Academy to its present position of power. Every episode is documented: there are over a thousand references, nearly all of them in Russian. It would be unfair to Vucinich to say he writes with detachment (who can be detached about tragedy?) but he does write with judicial care. His book is of course coloured by his convictions, but not by his prejudices.

Vucinich outlines the ancestry of the Academy. It began in 1725, when there was no higher education in Russia, and scholars had to be imported from abroad. It is no wonder that chauvinistic Russians came to regard it as a foreign implantation and, at

times of political unrest in Europe, as a source of subversion. "Dark forces" in the Academy were accused of closing its doors to Russian talent; this followed the rejection of Mendeleev's candidature for membership in 1880. There were periods of eclipse and recovery of its prestige. On the eve of the October Revolution it had pride of place among scientific institutions in Russia but (as Vucinich puts it) "it was more a symbolic expression of achievement in Russian science than a real workshop of new ideas". It was a Valhalla for the illustrious, not an inspiration for the young. This was the body inherited by the first Bolshevik government; under socialism it had to find a new identity if it was to survive.

Marxism in twentieth-century Russia, like the Church in mediaeval Europe, had to permeate every vein and capillary of thought and belief. The first task, therefore, was to harmonize the practice of science with the tenets of dialectical materialism. To this end, a Communist Academy was set up, aided by a Society of Militant Dialectical Materialists. They picked on members of the old guard in the Academy: one for flirting with Bergsonian vitalism, another for a supine acceptance of the work of Einstein, another for uncritical faith in Mendelian theory. Their sole constructive task was to propound a unified Marxist theory of science. In this task they failed. The Marxist theorists were split into feuding camps and, while they disputed among themselves, many scientific workers in the Academy of Sciences were able to keep a low profile and get on with their research. But they worked under perpetual anxiety and in grim poverty: "the famed mathematician A.A. Markov informed the Academy of Sciences that he could not attend the scholarly meetings because he did not own a wearable pair of shoes".

The government had the option of destroying the Academy or of casting it in a fresh mould. Fortunately Russian pride persuaded the government to reject the first option; the society whose membership had included Lomonosov, Euler, Markov, Pavlov and Vernadskii was too valuable to destroy. So they began the process of remoulding. There had to be emphasis on applied science; that was inevitable in the chaotic state of Russia in the 1920s. Lunacharskii, the minister responsible for education and cultural affairs, persuaded the government to embark on a policy of lavish support for the Academy. In 1925 it celebrated its 200th anniversary in optimistic festivity. Trotsky spoke to the assembled guests, one of whom, the British geneticist Bateson, wrote: "it was interesting to hear the faith that the advancement of science is a first duty of the State proclaimed by professional politicians".

The Academy had survived the October Revolution, but its optimism was soon dispelled. By 1929 a deliberately organized

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barrage of criticism descended upon it. The academicians had flagrantly violated the trust put in them by the State; "super-cilious mandarins of science" were holding up the sovietization of the Academy. It was hinted that it had become a centre for counter-revolutionary activity. By the end of 1929 there had been purges of the more outspoken academicians; one third of the persons elected between 1929 and 1931 were Marxists and the permanent secretary (a powerful office) was a stooge of the government. The Academy had been taken over by the Party.

The next phase in the Academy's history, until Stalin's death in 1953, is common knowledge. Vucinich calls it "the triumph of ideology". The Communist Academy had been incorporated in the Academy itself, "without", as Vucinich wryly says, "enriching it in any way". The sinister shadow of Lysenko eclipsed biology. Other sciences suffered, including physics, though there were a few disciplines, unintelligible to Marxist philosophers (crystallography was one of them) which managed to avoid attack. The social sciences were either suppressed or put into a framework as rigid as that of the mediaeval astronomers. Campaigns were mounted in the popular press on such recondite topics as quantum chemistry and resonance theory. At one conference a furious attack was made on the work of Pauling; at another there was a vilification of Western cultural anthropology. The pattern repeated itself: organized gangs in the audience "with no interest or competence in the pursuit of science" cheered the pre-ordained victors and taunted the pre-ordained villains.

And yet, in a way, it was not a "triumph of ideology". For behind the servile genuflections to Stalin, behind the abject confessions of deviation, many scientists kept faith with science, and good work was done. On the eve of the Second World War research was being done in physical chemistry by Semenov and Flerov; Kapitsa was working on superfluidity in helium II; Kol'tsov was discovering the function of macromolecules in heredity. It was a period of heroism, too. The veteran academician Priianishnikov (with whom I had long conversations when I was stationed in Moscow) "spent endless hours pleading with police authorities" to release colleagues from prison. To add to the complexity of the situation, the Academy was in fact very well financed in the 1940s. Because of the demands of the war, the ideological wrangles were for a time put aside. Vucinich notes the paradox: "Although deeply scarred by the reign of terror, the Academy entered the 1940s in a state of rapid rebuilding and with a generally optimistic outlook". And this, despite what he calls "annoying perturbations on the ideological front". The 220th anniversary of the Academy was celebrated in 1945, only a matter of weeks after the defeat of Germany. Bewildered guests were

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Peter Kapitsa (1894-1984) — kept faith with science.

brought in Soviet planes from a dozen countries and treated to interminable harangues, tedious formal sessions, and a banquet, attended by Stalin, on a scale lavish enough to have satisfied the Czars. But between the formalities Soviet scientists established — many of them for the first time — informal contact with their peers. After the birthday party the curtain came down again; cosmopolitanism remained a crime; the pressure to create a unique Soviet science remained a goal; demotion, exile, even death, remained occupational risks of intellectuals. But the Soviet government was far too shrewd to fall behind the West as exciting opportunities occurred in physics, cybernetics and biology. And so when Stalin died there was indeed an "empire of knowledge" ready to break the constraints imposed upon it for so many years.

The rate of recovery was astonishing.

Three years after Stalin's death there was a successful seminar on molecular biology, a subject Lysenko had banned from his institute. By 1965 Lysenko's last defender, Krushchev, had gone, and a commission was appointed which declared that the official biological doctrine established by Lysenko "was built on a combination of planned deceit, unfounded hopes, and transparent flaws in scientific premises". Miraculously the cult of anti-cosmopolitanism in science evaporated. But immense harm had been done and the State knew it, so there followed an intense phase of expansion and deliberate decentralization. Too much science had been concentrated in Moscow, so whole science cities were created east of the Urals and a massive programme to recruit students from the various republics was launched. The Academy, long under pressure to give emphasis to engineering and other applied sciences, reasserted its need to be free to pursue studies unrelated to immediate economic ends. The "quiet but stubborn resistance of a solid core of academicians" won through.

All this, and much more, is packed into Vucinich's remarkable book. It records a drama not only of Russian science, but of the Russian people. As I put the book down I recalled the words of the prosecutor Kirilovich in *The Brothers Karamazov*:

We are immoderate, we are an astonishing blend of good and evil . . . we are able to accommodate every possible contradiction . . . we are broad like Mother Russia herself; we find room for everything, we reconcile ourselves to everything. □

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Continental changes

Norman K. Grant

The Geochronology and Evolution of Africa.

By L. Cahen, N.J. Snelling, J. Delhal and J.R. Vail.

Clarendon: 1984. Pp.512. £60, \$110.

The Geochronology and Evolution of Africa is an extraordinary achievement. Unlike many other books in which one or more editors seek to link disparate contributions from various authors, the late Lucien Cahen and his co-authors and collaborators M. Bonhomme and D. Ledent have produced a text in which a rare and thorough scholarship shines through from beginning to end. In the book we have the latest expression of the deep connection of Europe with Africa, a connection which through colonial ties has led European geologists to become involved in and impassioned with the geology of Africa.

This book follows an earlier work by

Lucien Cahen and N.J. Snelling, *The Geochronology of Equatorial Africa* (North-Holland, 1966), but its roots go further back. In 1948 the late Arthur Holmes, who worked in Africa and collaborated with Cahen, urged that isotopic ages in combination with field observations on lithology and structure should be the basis for studies of the overwhelming diversity in the crystalline shield of Africa. Cahen pursued Holmes's strategies in regional geochronology until his own death in 1982. These strategies also carried within them the ambition of synthesizing and thus understanding more clearly the geology of Africa, as the influential papers by W.Q. Kennedy in 1964 and 1965 so clearly show.

Any review of the geochronology of Africa must face the problems that much of the information comes from samples not necessarily chosen for isotopic study or collected by geochronologists, and that results are often of poor analytical quality and based on a variety of decay constants. Cahen and his colleagues have addressed these difficulties by using the original isotopic measurements to re-calculate the ages

using modern decay constants, quoting MSWD values for all the re-calculated linear regressions. The introduction describes these fresh calculations, discusses how the re-calculated ages are used, and reviews new methods of age determination and interpretation.

The following 20 chapters describe the Precambrian geology of Africa, starting with the early Precambrian platform of southern Africa and ending with the Trans-Sahara mobile belt which extends from Algeria to Benin and Nigeria in the south. The chapters have a strong thematic quality which preserves the unity of the different aspects of the shield geology, and each of them is illustrated with maps and includes a review or synopsis.

The last three chapters begin with a review of the Phanerozoic igneous activity in Africa (which is closely connected to the thermal structure established during the late Precambrian Pan-African tectono-thermal event), followed by accounts of the succession of tectono-thermal events in Africa and the authors' conclusions concerning the evolution of the continent. Among these conclusions, the tectono-thermal and magmatic events in Africa are seen to have continued since early Archaean times with only short interruptions around 2,440–2,275 Myr and 1,740–1,425 Myr, this almost continuous history of events consisting of two similar sequences. The first began with the appearance of crustal stability around 2,500 Myr, followed by localized rifting and a sequence of tectono-thermal events which led into anorogenic granite plutonism down to 1,750 Myr. The second sequence began with local tectono-thermal events at c.1,400–c.1,300 Myr leading into several regional events through 1,185–1,140 Myr and c.950–c.600 Myr. This was followed by anorogenic granite plutonism until 425 Myr, after which the African shield gradually became destabilized by rifting from c.200 Myr to the present day.

By its nature this is not a book to read casually, yet the immense amount of information and the insightful and critical approach make it a fundamental resource for anyone with interests in Africa and the way regional geochronology leads to an understanding of its history. Geological cross-sections would have helped bring to life the details of the text and its numerous maps, and it is hard not to express some disappointment that the authors decided not to examine the ways in which the isotopic composition of strontium constrains African crustal evolution.

Yet the final word must be the sense of authenticity which comes from the text, an authenticity which will be apparent not only to those who have first-hand knowledge of Africa and its geology, but also to anyone who has come to value an understanding of its geological history. □

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All in the mind

David Singmaster

The Great Mental Calculators: The Psychology, Methods, and Lives of Calculating Prodigies Past and Present.

By Steven B. Smith.

Columbia University Press: 1983. Pp.374. \$29.50, £28.

PRODIGES occur in only a few fields: mathematics (usually in mental calculation), chess, music, memorization and languages. They intrigue us because their abilities are so unusual and they are often written about, though usually in the popular press where their freak value is well appreciated.

The present book is the first devoted entirely to calculating prodigies. It greatly expands and extends previous, more general works such as A. Binet's *Psychologie des Grands Calculateurs et Joueurs d'Echecs* (1894), F. Barlow's *Mental Prodigies* (1951) and R. Tocquet's *The Magic of Numbers* (1960). Professor Smith's subtitle is an accurate description of the contents of his book, though I find it convenient to consider the three main sections in reverse order.

The final section, 'Lives', gives detailed descriptions of 20 calculators and brief accounts of 21 more. A further 15 or so are mentioned elsewhere in the text. Smith is especially strong on contemporary calculators; about 12 of the people mentioned may currently be alive. Such a figure indicates that the incidence is about one in several hundred million, that is between 5.6 and 6.0 standard deviations from the mean. This seems as good a definition of prodigality as any, though earlier estimates varied from one in a thousand to one in a million.

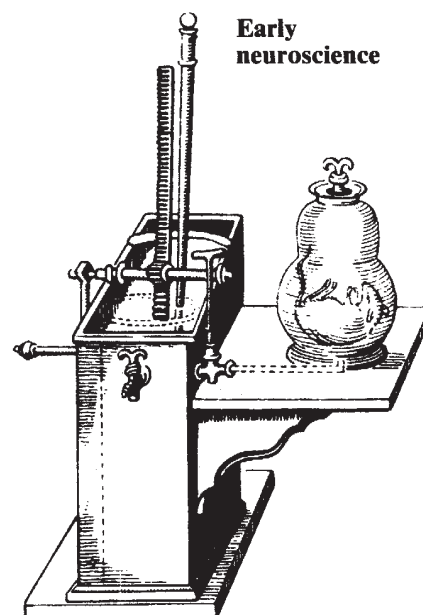
The two most notable prodigies covered here are George Bidder and Alexander Aitken. Bidder (1806–1878) was the son of a Devon stonemason. He discovered multiplication by arranging objects in a rectangle, and by the age of nine could easily multiply two six-digit numbers mentally. He became an outstanding engineer in that era of great engineers, being proposer and engineer of the Victoria Docks in London, a founder of the Electric Telegraph Company and President of the Institution of Civil Engineers. His abilities as an expert witness were legendary — one opposing counsel asked for him to be barred since "nature had endowed him with particular qualities that did not place his opponents on a fair footing".

Bidder was especially good on logarithms and compound interest. And two days before his death, he mentally calculated the frequency of red light from the speed of light (then taken as 190,000 miles s⁻¹) and the fact that there are 36,918 wavelengths per inch, obtaining the result 444,433,651,200,000.

Alexander Aitken (1895–1967) was born in New Zealand, the son of a grocer's assistant. He displayed no unusual talents until he was 13, when a master remarked that squares could be computed using $a^2 = (a + b)(a - b) + b^2$. From then he spent the next four years practising mental calculation; in 1916, in a remarkable feat, he recalled all the details of men in a platoon when the roll-book was lost in a raid. Aitken eventually became professor of mathematics at Edinburgh University and a Fellow of the Royal Society. His speciality was the extraction of non-integral roots (for example $\sqrt{51} = 7.141428428557$, where the last two places should be 43) and finding decimal expansions of rationals (for example the 96 figures of the expansion of $1/97$).

Both Bidder and Aitken stand out for two further reasons. Each wrote extensive descriptions of his methods, and each made wide use of his skills in his professional life, applying mathematics to develop further calculational methods and to extend the range of problems susceptible to mental calculation.

The second section, 'Methods', is the briefest and covers the techniques actually used by calculators. Though the mathematics is usually straightforward, the application is often remarkably ingenious and skilfully arranged to minimize memory load. Using some of these ideas and a little practice, I think anyone could mentally square a two-digit number and perhaps multiply two two-digit numbers. More interesting are the methods used for non-integral roots, logarithms, compound



Robert Boyle's experimental apparatus in which air was evacuated from a chamber containing an animal, thus asphyxiating the animal and showing that "there is in the Air a little vital Quintessence". The illustration is taken from Mary A.B. Brazier's *A History of Neurophysiology in the 17th and 18th Centuries*, published by Raven price \$81.

interest and factoring, though these were only used by more sophisticated calculators.

In the first section of the book — dealing with psychology — Smith discusses the various factors relating to mental calculation, drawing on the mathematical and biographical material summarized in his later sections. Here he presents a thesis that mental calculation and language are the same mental skill (Smith himself is a linguist). He makes a reasonable case for this, though it does lead him away from the main stream of his material.

I found two other themes in this first section. First, there has been surprisingly little experimental work with prodigies, and there seems to be little agreement as to what should be tested and how. Second, many of the common conceptions about prodigies are false.

For example, it is widely thought that calculators have a photographic memory and “see” numbers. Binet was the first to discover that this is not necessarily the case. His subject definitely heard numbers and had not learned written numbers until after his mental powers were developed. About half the prodigies are “auditors” and there is a blind prodigy who feels numbers. In addition it is clear that while the memory of prodigies is good for what they are interested in, it is often not exceptional in other ways; indeed musicians, actors, linguists and so on routinely perform more remarkable feats of memory. All the calculators agree that some mental calculation and memorization can be achieved by anyone with sufficient interest.

Also there is the myth that exceptional mental powers are the prerogative of extreme youth. Several prodigies in fact started in their teens and three contemporary ones started in their twenties. Almost all retained their powers throughout life. And although there is a disproportionate number of *idiots savants* and of geniuses among them — about eight of each among the 70-odd cited by Barlow, Tocquet and Smith — the remaining 50 or so prodigies appear to have been fairly normal or successful in life.

Finally, there is one commonly held belief that is valid — most prodigies are indeed male (only six females are mentioned by Smith). Heredity does not seem to be a factor, the only cases where mental calculation runs in a family being where there is clear influence among the calculators.

Professor Smith has written a quite fascinating book and has also done a useful job in bringing together all of this information. The study of prodigies has much to offer the disciplines of psychology, mathematics and linguistics, and this book will have performed a valuable service if it provokes research into their unusual powers. □

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Dangerous talk

Alastair Hay

Workers at Risk: Voices from the Workplace.

By Dorothy Nelkin and Michael S. Brown.

University of Chicago Press: 1984. Pp.222. \$20, £17.

“They have government agencies that protect you if you buy a defective washing machine or if your car doesn’t steer straight, but nobody’s interested in chemicals”.

“This government is obviously disengaging itself wholesale from worker safety”.

“OSHA [the US Occupational Safety and Health Administration] is what we have to work with, and OSHA is nothing. . . . I realized how ineffective OSHA really is”.

“I wouldn’t dare call OSHA. Our jobs would be in jeopardy. We would be gone within a month”.

“You have to sneak just for your own safety”.

“The company always knows when OSHA’s coming because when OSHA’s coming we never have the same fumes as we have when OSHA’s not coming”.

THESE are the voices of disenchanted workers in the United States, taken from Dorothy Nelkin and Michael Brown’s innovative and perceptive book. There are many who will object to it — captains of industry will find it hurtful; and those in government charged with the responsibility for workers’ safety and health will be stung by the accusations that not only are they doing little about improving safety, but that they are actually making things worse by weakening some of the legislation on the subject.

These are harsh judgements but they are no less valid because they are made by people with a vested interest in protecting their own health. It is rare to find a book which gives workers the opportunity to voice their misgivings. More usually such comments are sanitized in some government report or rendered even more anonymous as just another statistic. The value of *Workers at Risk* is that its subjects are recognizable. They are men and women employed in all manner of jobs — chemical operators, laboratory technicians, railroad workers, orchard workers, painters, sculptors, deckhands are but a few — where they come into contact with chemicals. If they have one complaint in common, it is that they do not know enough about the danger of the materials they work with.

In some ways these individuals are the dispossessed — they have nothing to offer but their labour and in a recession even that comes into jeopardy. Fighting for better safety might make them marked individuals, and the first to be fired when redundancies are made. As the book makes clear, workers in plants that are unionized have a much better chance of implementing changes than their peers in non-union

plants. In the former, the whole of the workforce can be relied on to demand changes, whereas in the latter it is up to individuals with the consequently greater threat of being victimized or fired.

There is resentment too over the actions of the workers’ watchdog, the Occupational Safety and Health Administration. Charged under the 1970 Occupational Safety and Health Act with setting standards and policing them, the agency has been less active recently than it was in its formative years, particularly when Dr Eula Bingham, a well known toxicologist, was OSHA assistant secretary from 1977 to 1980. Bingham gave the agency a sense of purpose. Mere tinkering with the system was not her idea of how to prevent occupational illness, and she introduced new health regulations which were supported by the labour unions but, predictably, opposed by industry.

President Reagan’s appointee to head OSHA in 1980, Thorne Auchter, a Florida construction contractor, has a different philosophy. He believes the industry can protect its workforce more efficiently without government interference. To this end, Auchter revoked or reinterpreted many of the regulations introduced in the 1970s. As a result inspections are down by a third, citations for violations are down by at least that amount and the backlog of worker complaints has risen by 105%.

None of these developments are reassuring to the people represented in the book. As a computer assembler put it:

. . . who are we going to fall back on, especially those workers who don’t have a union to protect them? Our only alternative is supposedly our government, but it doesn’t seem to be our’s anymore, so we’re going to have to find new ways of protecting ourselves.

Such words may sound like a threat of anarchy, but that would be the wrong interpretation. The lesson is not that industry will suffer unduly — the workers still want their jobs — but that it can be made safer. And if the government agencies are not going to provide the assistance they could, then individuals will have to find out information for themselves. More people than ever recognize the long-term health effects of exposure to chemicals and this widespread, critical awareness is certain to increase. Although critics dismiss such fears as “chemophobia”, there is no getting away from the fact that people are worried, genuinely so, about exposure to chemicals.

Toxicologists may find some of the arguments in the book specious, and some of the fears unfounded, but it is hardly the fault of the men and women concerned. Most lack the knowledge which would reassure them and lead to a change in habits. *Workers at Risk* is a provocative book which will be invaluable in spreading that message. □

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Physics

Stochastic Quantum Mechanics and Quantum Spacetime: A Consistent Unification of Relativity and Quantum Theory Based on Stochastic Spaces. By EDUARD PRUGOVEČKI. Reidel: 1984. Pp.302. ISBN 90-277-1617-X. \$48.50.

Supersymmetry and Supergravity 1983. Proceedings of the XIXth Winter School and Workshop of Theoretical Physics. B. MILEWSKI (ed.). Wiley: 1983. Pp.578. Hbk ISBN 9971-950-23-5; pbk ISBN 9971-950-97-9. Hbk £49.50.

Theory of Dispersed Multiphase Flow. RICHARD E. MEYER (ed.). Academic: 1983. Pp.388. ISBN 0-12-493120-0. \$28.

Chemistry

Adsorption on and Surface Chemistry of Hydroxyapatite. DWARIKA N. MISRA (ed.). Plenum: 1984. Pp.179. ISBN 0-306-41516-X. Np.

Advances in Chromatography, Vol. 23. J. CALVIN GIDDINGS *et al.* (eds). Marcel Dekker: 1984. Pp.272. ISBN 0-8247-7075-7. SwFr. 140.

Chemical Instabilities. G. NICOLIS and F. BARAS (eds). Reidel: 1984. Pp.420. ISBN 90-277-1705-2. Dfl. 155, \$58.

Chemical Principles 4th Edn. By RICHARD E. DICKERSON *et al.* Addison-Wesley: 1984. Pp.930. ISBN 0-8053-2422-4. £31.45.

Chemistry of the Elements. By N. N. GREENWOOD and A. EARNSHAW. Pergamon: 1984. Pp.1542. ISBN 0-08-022057-6. £19.50, \$34.95.

Deactivation of Catalysts. By R. HUGHES. Academic: 1984. Pp.265. ISBN 0-12-360870-8. £30.00, \$42.00.

Fracture Mechanics of Polymers. By J. G. WILLIAMS. Ellis Horwood: 1984. Pp.302. ISBN 0-85312-685-2. Np.

Open Tubular Column Gas Chromatography: Theory and Practice. By MILTON L. LEE *et al.* Wiley: 1984. Pp.445. ISBN 0-471-88024-8. £45.60.

Reactive Molecules: The Neutral Reactive Intermediates in Organic Chemistry. By CURT WENTRUP. Wiley: 1984. Pp.333. ISBN 0-471-87639-9. Np.

Computer Science

Database Management in Science and Technology: A CODATA Sourcebook on the Use of Computers in Data Activities. JOHN R. RUMPLE Jr and VIKTOR E. HAMPEL (eds). North-Holland: 1984. Pp.263. ISBN 0-444-86865-8. Np.

Introduction to Interval Computations. Computer Science and Applied Mathematics. By GÖTZ ALEFELD and JÜRGEN HERZBERGER. Academic: 1983. Pp.333. ISBN 0-12-049820-0. \$49.50.

Introduction to Numerical Computation, 2nd Edn. By JAMES S. VANDERGRAFT. Academic: 1983. Pp.372. ISBN 0-12-711356-8. \$29.50.

Technology

Advances in Biochemical Engineering/Bio-technology. Immobilized Biocatalysts *Saccharomyces* Yeasts Wastewater Treatment. S. AIBA *et al.* (eds). Springer-Verlag: 1984. Pp.150. ISBN 3-540-12860-3/0-387-12860-3. DM 78; \$30.30.

Biotechnology Advances: Research and Patent Abstracts. M. MOO-YOUNG *et al.* (eds). Pergamon: 1984. Pp.389. ISBN 0-08-031708-1. £37.50, \$60.00.

Energy Resources through Photochemistry and Catalysis. MICHAEL GRÄTZEL (ed.). Academic: 1983. Pp.573. ISBN 0-12-295720-2. \$59.50.

Engineering Risk. Institution of Professional Engineers, PO Box 12-241, Wellington, New Zealand: 1983. Pp.95. Pbk ISBN 0-9597694-0-4. \$NZ11.95.

Man and Technology: The Social and Cultural Challenge of Modern Technology. BRUCE M. ADKINS (ed.). Cambridge Information and Research Services: 1984. Pp.300. Pbk ISBN 0-905332-30-X. Pbk £15.

Microcomputers and Laboratory Instrumentation. By DAVID J. MALCOLME-LAWES. Plenum: 1984. Pp.246. ISBN 0-306-41668-9. Np.

Biological Sciences

Minimal Residual Disease in Acute Leukemia: Developments in Oncology. B. LÖWENBERG and A. HAGENBEEK (eds). Martinus Nijhoff: 1984. Pp.401. ISBN 0-89838-630. Dfl. 165, £41.95.

Mitochondria 1983: Nucleo-Mitochondrial Interactions. R.J. SCHWEYEN *et al.* (eds). Walter de Gruyter: 1983. Pp.648. ISBN 3-11-0098717. DM 240.

The Molecular Biology of Adenoviruses 1. Current Topics in Microbiology and Immunology, Vol. 109. WATER DOERFLER (ed). Springer-Verlag: 1983. ISBN 3-540-13034-9/0-387-13034-9. Dm 118, \$45.80.

Molecular Pathology of Nerve and Muscle: Noxious Agents and Genetic Lesions. ANTONY D. KIDMAN *et al.* (eds). Humana Press: 1984. Pp.432. ISBN 0-89603-057-1. \$59.50.

The Mollusca, Vol. 5. Physiology, Part 2. A.S.M. SALEUDDIN and K.M. WILBUR (eds). Academic: 1983. Pp.500. ISBN 0-12-751405-8. \$62.

Mouse Mammary Tumor Virus. Current Topics in Microbiology and Immunology, Vol. 106. P.K. VOGT and H. KOPROWSKI (eds). Springer-Verlag: 1983. Pp.103. ISBN 3-540-12828-X/0-387-12828-X. DM 58, \$22.50.

Myocardial Infarction and Cardiac Death. E. MARGULIES (ed.). Academic: 1983. Pp.218. ISBN 0-12-471350-5. \$32.50.

Neuroethology. By JEFFREY M. CAMHI. Blackwell Scientific: 1984. Pp.416. ISBN 0-87893-075-2. £24.

Neuromuscular Diseases. GEORGES SERRATRICE *et al.* (eds). Raven: 1983. Pp.630. ISBN 0-89004-970-X. \$118.

Neutrons in Biology. Basic Life Sciences, Vol. 27. BENNO P. SCHOENBORN (ed.). Plenum: 1984. Pp.462. ISBN 0-306-41508-9. \$59.50.

Nutrition and Feeding Strategies in Protozoa. By B. NISBET. Croon Helm: 1984. Pp.280. ISBN 0-7099-1800-3. £19.95.

Nutritional Adaptation of the Gastrointestinal Tract of the Newborn. N. KRETCHMER and A. MINKOWSKI (eds). Raven: 1983. Pp. 239. ISBN 0-89004-905-X. \$37.

Occupational Lung Disease. J. BERNARD L. GEE *et al.* (eds). Raven: 1983. Pp.296. ISBN 0-89004-900-9. \$49.

Peptide and Protein Reviews, Vol. 2. MILTONT.W. HEARN (ed.). Marcel Dekker: 1984. Pp.296. ISBN 0-8247-7135-4. SwFr. 146.

Physiology of the Human Body, 6th Edn. By ARTHUR C. GUYTON. Saunders College Publishing: 1984. Pp.691. ISBN 0-03-058339-X. Np.

Plant and Fungal Toxins. Handbook of Natural Toxins, Vol.1. RICHARD F. KEELER and ANTHONY T. TU (eds). Marcel Dekker: 1983. Pp.934. ISBN 0-8247-1893-3. SwFr. 392.

Plant Poisoning in Animals: A Bibliography from the World Literature 1960-1979. Commonwealth Agricultural Bureaux: 1984. Pp.40. Pbk ISBN 0-85198-525-4. £12, \$25.20.

Primary Immunodeficiency Diseases. R.J. WEDWOOD, F.S. ROSEN and N.W. PAUL (eds). Alan R. Liss: 1983. Pp.388. ISBN 0-8451-1054-3. £78.

Princes and Peasants: Smallpox in History. By DONALD R. HOPKINS. University of Chicago Press: 1983. Pp.380. ISBN 0-226-35176-9. £21.25.

Proceedings of the First International Symposium on Light and Oxygen Effects on the Eye. S.D. VARMA and S. LERMAN (eds). IRL Press: 1984. Pp.278. Pbk ISBN 0-904147-62-2. Pbk £20, \$40.

Recent Advances in Male Reproduction: Molecular Basis and Clinical Implications. Sero Symposium Publications, Vol. 7. R. D'AGATA *et al.* (eds). Raven: 1983. Pp.350. ISBN 0-89004-918-1. \$61.

Reproduction in Mammals, 2nd Edn. Hormonal Control of Reproduction, Book 3. C.R. AUSTIN and R.V. SHORT (eds). Cambridge University Press: 1983. Pp.244. Hbk ISBN 0-521-25637-2; pbk ISBN 0-521-27594-6. Hbk £22.50; pbk np.

Reproductive and Developmental Toxicity of Metals. T.W. CLARKSON, G.F. NORDBERG and P.R. SAGER (eds). Plenum: 1983. Pp.845. ISBN 0-306-41396-5. \$115.

Retroviruses 2. Current Topics in Microbiology and Immunology, Vol. 107. P.K. VOGT and H. KOPROWSKI (eds). Springer-Verlag: 1983. Pp.180. ISBN 3-540-12384-9/0-387-12384-9. DM 96, \$37.30.

Scleroderma Canker of Conifers. Forestry Sciences. PAUL D. MANION (ed.). Martinus Nijhoff/Dr W. Junk: 1984. Pp.273. ISBN 90-247-2912-2. £28.50, \$43.

Sexual Differentiation: Basic and Clinical Aspects. Sero Symposium Publications from Raven Press, Vol. 11. Raven: 1984. Pp.384. ISBN 0-89004-316-7. \$53.50.

Special Topics in Endocrinology and Metabolism, Vol. 5. MARGO P. COHEN and PIERO P. FOA (eds). Alan R. Liss: 1984. Pp.310. ISBN 0-8451-0704-0. £37.

Structure and Function in Excitable Cells. D.C. CHANG *et al.* (eds). Plenum: 1983. Pp. 499. ISBN 0-306-41338-8. \$65.

Synergetics of the Brain. Springer Series in Synergetics, Vol. 23. E. BASAR *et al.* (eds). Springer-Verlag: 1983. Pp. 377. ISBN 3-540-12960-X/0-387-12960-X. DM 88, \$34.20.

The Target of Penicillin: The Murein Sacculus of Bacterial Cell Walls' Architecture and Growth. R. HAKENBECK *et al.* (eds). Walter de Gruyter: 1983. Pp.663. ISBN 3-11009705-2. DM 180.

Time Resolved Fluorescence Spectroscopy in Biochemistry and Biology. NATO Advanced Science Institute Series, Vol. 69. R.B. CUNDALL and R.E. DALE (eds). Plenum: 1983. Pp.778. ISBN 0-306-41476-7. \$110.

Topics in Photomedicine. KENDRIC C. SMITH (ed.). Plenum: 1984. Pp.403. ISBN 0-306-41510-0. \$65.

Toxins as Tools in Neurochemistry. F. HUCHO and Y.A. OVCHINNIKOV (eds). Walter de Gruyter: 1983. Pp.368. ISBN 3-11-009593-9. DM 180.

Treasure of the Sea: Marine Life of the Pacific Northwest. By JAMES CRIBB. Oxford University Press: 1984. Pp.96. ISBN 0-19-540418-1. £21.

Trees and Networks in Biological Models. By N. MACDONALD. Wiley: 1983. Pp. 215. ISBN 0-471-10508-2. £18, \$33.75.

Tumor Viruses and Differentiation. E.M. SCOLNICK and A.J. LEVINE (eds). Alan R. Liss: 1983. Pp. 476. ISBN 0-8451-2604-0. \$88.

Tutorials in Learning and Memory: Essays in Honor of Gordon Bower. JOHN R. ANDERSON and M. KOSSLYN (eds). W.H. Freeman: 1984. Pp.264. Hbk ISBN 0-7167-1570-8; pbk ISBN 0-7167-1571-6. Hbk £21.95; pbk £11.95.

Urologic Cancer: A Multidisciplinary Perspective. M.B. GARNICK and J.P. RICHIE (eds). Plenum: 1983. Pp.275. ISBN 0-306-41473-2. \$35.

Vagal Nerve Function: Behavioral and Methodological Considerations. J.G. KRAL *et al.* (eds). Elsevier: 1984. Pp.345. ISBN 0-444-80550-8. \$40, Dfl. 104.

Vascular Neuroeffector Mechanisms: 4th International Symposium. J.A. BEVAN *et al.* (eds). Raven: 1983. Pp. 455. ISBN 0-89004-738-3. \$93.

Vertebrate Natural History. By MARY F. WILLSON. Saunders College Publishing: 1984. Pp.621. ISBN 0-03-061804-5. Np.

The Wildlife and Nature Photographer's Field Guide. By MICHAEL FREEMAN. Croom Helm: 1984. Pp.223. ISBN 0-7099-1025-8. £8.95.

Applied Biological Sciences

Advances in Biotechnological Processes, Vol. 2. AVSHALOM MIZRAHI and ANTONIUS L. VAN WEZEL (eds). Alan R. Liss: 1983. Pp.612. ISBN 0-8451-2607-5. £58.

Advances in Cancer Research, Vol. 40. G. KLEIN and S. WEINHOUSE (eds). Academic: 1983. Pp.441. ISBN 0-12-006640-8. \$54.

Advances in Human Genetics, Vol. 13. H. HARRIS and K. HIRSCHHORN (eds). Plenum: 1983. Pp.312. ISBN 0-306-41431-7. \$42.50.

Advances in Pain Research and Therapy: Neural Mechanisms of Pain, Vol. 6. LAWRENCE KRUGER and JOHN C. LIEBESKIND (eds). Raven: 1983. Pp.384. ISBN 0-89004-921-1. \$61.

Antibodies: Their Structure and Function. Outline Studies in Biology. By M.W. STEWARD. *Chapman and Hall*: 1984. Pp.96. ISBN 0-412-25640-1. Pbk £3.25.

Application of Biological Markers to Carcinogen Testing. H.A. MILMAN and S. SELL (eds). *Plenum*: 1983. Pp.516. ISBN 0-306-41490-2. \$69.50.

Application of Physics to Medicine and Biology. G. ALBERI, Z. BAJZER and P. BAXA (eds). *World Scientific*: 1983. Pp.663. ISBN 9971-950-42-1; pbk ISBN 9971-950-43-X. Hbk £50, \$70; pbk np.

Aspects of Human Genetics: With Special Reference to X-Linked Disorders. C. SAN ROMÁN and A. MCDERNOTT (eds). *Karger*: 1984. Pp.156. SwFr.98, Dm 117, \$58.75.

Atherosclerosis: Mechanisms and Approaches to Therapy. N.E. MILLER (ed.). *Raven*: 1983. Pp.237. ISBN 0-89004-898-3. \$56.

Biochemical Engineering III. Annals of the New York Academy of Sciences, Vol. 413. K. VENKATASUBRAMANIAN *et al.* (eds). *The New York Academy of Sciences*: 1983. Pp.562. Hbk ISBN 0-89766-221-0, pbk ISBN 0-89766-221-0. Pbk \$112.

Biological Response Modifiers: Subcommittee Report Monograph 63. PETER GREENWALD and ELIZABETH K. WEISBURGER (eds). *US Government Printing Office*: 1984. \$10.

Biological Rhythms and Medicine: Cellular, Metabolic, Physiopathologic, and Pharmacologic Aspects. Topics in Environmental Physiology and Medicine. By ALAIN REINBERG and MICHAEL H. SMOLENSKY. *Springer-Verlag*: 1983. Pp.305. ISBN 3-540-90791-2/0-387-90791-2. DM 185, \$71.80.

Biotechnology: A Comprehensive Treatise in 8 Volumes, Vol. 5. G. REED (ed.). *Verlag-Chemie*: 1983. Pp.631. ISBN 3-527-25767-5/0-89573-045-6. DM 425.

Biotechnology & Genetic Engineering Review Vol. 1. G.E. RUSSELL (ed.). *Intercept*, PO Box 2, Ponteland, Newcastle upon Tyne, England: 1984. Pp.437. ISBN 0-946707-01-4. £40, \$79.

Brain Tumors in Children: Principles of Diagnosis and Treatment. The International Review of Child Neurology. By MICHAEL E. COHEN and PATRICIA KRESSEL DUFFNER. *Raven*: 1984. Pp.378. ISBN 0-89004-935-1. \$56.

Calcium Antagonists and Cardiovascular Disease. Perspectives in Cardiovascular Research, Vol. 9. L.H. OPIE (ed.). *Raven*: 1983. Pp.397. ISBN 0-89004-967-X. \$40.

Cancer Prevention in Clinical Medicine. GUY R. NEWELL (ed.). *Raven Press*: 1983. Pp.257. ISBN 0-89004-822-3. \$55.

Cardiovascular Pharmacology, 2nd Edn. MICHAEL J. ANTONACCIO (ed.). *Raven*: 1983. Pp.592. ISBN 0-89004-872-X. \$49.

The Chemotherapy of Psoriasis. International Encyclopedia of Pharmacology and Therapeutics Section 110. H.P. BADEN (ed.). *Pergamon*: 1984. Pp.314. ISBN 0-08-029823-0. £40, \$80.

Clinical Aspects of Essential Hypertension, Vol. 1. Handbook of Hypertension. J.I.S. ROBERTSON (ed.). *Elsevier*: 1983. Pp.517. ISBN 0-444-90307-0. Dfl. 211, \$86.

Clinical Aspects of Secondary Hypertension, Vol. 2. Handbook of Hypertension. J.I.S. ROBERTSON (ed.). *Elsevier*: 1983. Pp.333. ISBN 0-444-90308-9. Dfl. 188, \$79.

Clinical In Vitro Fertilization. CARL WOOD and ALAN TROUNSON (eds). *Springer-Verlag*: 1984. Pp.212. ISBN 3-540-12812-3/0-387-12812-3. DM 85, £22.

The Clinical Research Process in the Pharmaceutical Industry. Drugs and the Pharmaceutical Sciences, Vol. 9. GARY M. MATOREN (ed.). *Marcel Dekker*: 1984. Pp.576. ISBN 0-8247-1914-X. SwFr. 163.

Cochlear Implants in Clinical Use. Advances in Audiology, Vol. 2. W.D. KEIDEL and P. FINKENZELLER (eds). *Karger*: 1984. Pp.176. ISBN 3-8053-7271-2. SwFr. 140, DM 168, \$84.

Commentaries on Research in Breast Disease, Vol. 3. R.D. BULBROOK and D.J. TAYLOR (eds). *Alan R. Liss*: 1983. Pp.216. ISBN 0-8451-1902-8. \$42.

Complications in Neurosurgery I. A.M. LANDOLT (ed.). *Karger*: 1984. Pp.172. ISBN 3-8053-6991-7. SwFr. 98, DM 117, \$58.75.

Computed Tomography of the Thorax. DAVID P. NAIDICH *et al.* (eds). *Raven*: 1983. Pp.326. ISBN 0-89004-982-3. \$81.

Continuous Transcutaneous Blood Gas Monitoring. Reproductive Medicine Series, Vol. 5. R. HUCH and A. HUCH (eds). *Dekker*: 1983. Pp.872. ISBN 0-8247-1794-5. SwFr. 254.

The Control of Tumour Growth and its Biological Bases. Developments in Oncology. W. DAVIS *et al.* (eds). *Martinus Nijhoff*: 1984. Pp.466. ISBN 0-89838-603-9. Dfl. 200, \$77, £50.75.

Crop Breeding: A Contemporary Basis. P.B. VOSE and S.G. BLIXT (eds). *Pergamon*: 1983. Pp.443. ISBN 0-08-025505-1. £50, \$90.

Developmental Pharmacology. Progress in Clinical and Biological Research, Vol. 135. STUARTS M. MACLEOD *et al.* (eds). *Alan R. Liss*: 1983. Pp.450. ISBN 0-8451-0135-8. £60.

Developmental Processes in Normal and Diseased Muscle: Experimental Biology and Medicine Vol. 9. H.M. EPPENBERGER and J.C. PERRIARD (eds). *Karger*: 1984. Pp.293. ISBN 3-8055-3765-4. SwFr. 190, DM 228, \$114.

Diabetes Mellitus: Etiopathogenesis and Metabolic Aspects. F. BEFIORE *et al.* (eds). *Karger*: 1984. Pp.282. ISBN 3-8055-3771-9. SwFr. 212, DM 254, \$127.

Difficult Decisions in Medical Ethics. Progress in Clinical and Biological Research, Vol. 139. DOREEN GANOS *et al.* (eds). *Alan R. Liss*: 1983. Pp.254. ISBN 0-8451-0139-0. £29.

Drug Metabolism and Drug Toxicity. JERRY R. MITCHELL and MARJORIE G. HORNING (eds). *Raven*: 1983. Pp.436. ISBN 0-89004-997-1. \$97.

Drugs, Neurotransmitters, and Behavior. Vol. 18. Handbook of Psychopharmacology. LESLIE L. IVERSEN *et al.* (eds). *Plenum*: 1984. Pp.532. ISBN 0-306-41415-5. £65.

Endocrine Mechanisms in Fertility Regulation. G. BENAGIANO and E. DICZFALUSY (eds). *Raven*: 1983. Pp.366. ISBN 0-89004-464-3. \$67.

The Epidemiology of Cancer. GEOFFREY J. BOURKE (ed.). *Croom Helm*: 1984. Pp.373. ISBN 0-7099-0670-6. £19.95.

Epidemiology of Natural Disasters. Vol. 5. Contributions to Epidemiology and Biostatistics. By J. SEAMAN. *Karger*: 1984. Pp.177. ISBN 3-8055-3779-4. SwFr. 148, DM 177, \$88.75.

Evoked Potentials in Clinical Medicine. By K.H. CHIAPPA. *Raven*: 1983. Pp.352. ISBN 0-89004-777-4. \$42.50.

Experimental and Clinical Interventions in Aging. RICHARD F. WALKER and RALPH L. COOPER (eds). *Marcel Dekker*: 1983. Pp.448. ISBN 0-8247-7012-9.

Exploring the Heart: Discoveries in Heart Disease and High Blood Pressure. By JULIUS H. COMROE. *W.W. Norton*: 1983. Pp.348. ISBN 0-393-01708-7. Np.

Etrahepatic Drug Metabolism and Chemical Carcinogenesis. J. RYDSTRÖM *et al.* (eds). *Elsevier*: 1984. Pp.630. ISBN 0-444-80538-9. Dfl. 259, \$110.25.

Farm Animals: Husbandry, Behavior and Veterinary Practice. By MICHAEL W. FOX. *University Park Press*: 1984. Pp.285. ISBN 0-8391-1769-8. Np.

Gaseous Air Pollutants and Plant Metabolism. By M.J. KOZIOE and F.R. WHATLEY. *Butterworths*: 1984. Pp.466. ISBN 0-408-11152-6. £50.

Genetic Control of Environmental Pollutants. Basic Life Sciences, Vol. 28. GILBERT S. OMENN and ALEXANDER HOLLAENDER (eds). *Plenum*: 1984. Pp.408. ISBN 0-306-41624-7. \$55.

Genetic Engineering Applications to Agriculture. LOWELL D. OWENS (ed.). *Granada Publishing*, 8 Grafton Street, London W1: 1984. Pp.326. ISBN 0-246-11947-0. £25.

Genotoxicology of N-Nitroso Compounds. Topics in Chemical Mutagenesis. T.K. RAO *et al.* (eds). *Plenum*: 1984. Pp.271. ISBN 0-306-41445-7. Np.

Hormonally Defined Media: A Tool in Cell Biology. G. FISCHER and R.J. WIESER (eds). *Springer-Verlag*: 1983. Pp.455. ISBN 3-540-12668-6/0-387-12668-6. DM 78, \$30.30.

Hyperthermia and Radiation Therapy/Chemotherapy in the Treatment of Cancer. Frontiers of Radiation Therapy and Oncology. J.M. VAETH (ed.). *Karger*: 1983. Pp.196. ISBN 3-8055-3560-0. SwFr. 164, DM 196, \$98.25.

Immunobiology of Transplantation, Cancer and Pregnancy. P.K. RAY (ed.). *Pergamon*: 1983. Pp.445. ISBN 0-08-025994-4. \$65.

Immunomodulation: New Frontiers and Advances. H. HUGH FUDENBERG *et al.* (eds). *Plenum*: 1984. Pp.458. ISBN 0-306-41493-7. \$65.

Interferential Therapy. By BRENDA SAVAGE. *Faber & Faber*: 1984. Pp.117. Pbk ISBN 0-571-13202-2. Pbk £2.95.

Intervention in the Aging Process, Part A: Quantitation, Epidemiology and Clinical Research. Modern Aging Research, Vol. 3A. Alan R. Liss: 1983. Pp.360. ISBN 0-8451-2302-5. £40.

Intervention in the Aging Process, Part B: Basic Research and Preclinical Screening. Volume 3B, Modern Aging Research. WILLIAM REGELSON and F. MAROTT SINEX (eds). *Alan R. Liss*: 1983. Pp.360. ISBN 0-8451-2303-3. £46.

An Introduction to Animal Breeding, 2nd Edn. By JOHN C. BOWMAN. *Edward Arnold*: 1984. Pp.81. Pbk ISBN 0-7131-2880-1. Pbk £3.35.

Liver Cirrhosis. Frontiers of Gastrointestinal Research, Vol. 8. P. GENTILINI and M.U. DIANZANI (eds). *Karger*: 1984. Pp.280. ISBN 3-8055-3724-7. SwFr. 149, DM 178, £89.25.

Proteases: Potential Role in Health and Disease. Advances in Experimental Medicine and Biology, Vol.167. WALTER H. HORL and AUGUST HEIDLAND (eds). *Plenum*: 1984. Pp.591. ISBN 0-306-41488-0. Np.

Proteinase Inhibitors: Medical and Biological Aspects. NOBUHIKO KATUNUMS *et al.* (eds). *Springer-Verlag*: 1983. Pp.318. ISBN 3-540-12770-4/0-387-12770-4. DM 118, \$45.80.

Proteins in Body Fluids, Amino Acids, and Tumor Markers: Diagnostic and Clinical Aspects. S.E. RITZMANN and L.M. KILLINGSWORTH (eds). *Alan R. Liss*: 1983. Pp.506. ISBN 0-8451-2802-7. £34.

Radiation Carcinogenesis: Epidemiology and Biological Significance. Progress in Cancer Research and Therapy, Vol.26. JOHN D. BOICE Jr and JOSEPH F. FRAUMENI Jr. (eds). *Raven*: 1983. Pp.509. ISBN 0-89004-907-6. \$93.

Radionics. R.J. BLOOMFIELD. *Information and Study Centre for Alternative Medicine*, 64, Bower Mount Road, Maidstone, Kent, England: 1984. Pp.30. £1.25.

The Rat as Animal Model in Breast Cancer Research. Developments in Oncology. By MATTHEW J. VAN SWIETEN. *Martinus Nijhoff*: 1983. Pp.292. ISBN 0-89838-624-1. Dfl.140, \$52, £35.50.

Recent Advances in Obesity and Diabetes Research, Vol.8. N. MELCHIONI and D.L. HORWITZ (eds). *Raven Press*: 1984. Pp.414. ISBN 0-89004-969-6. \$86.

Recent Developments in Alcoholism, Vol.2. Learning and Social Models: Alcohol and the Liver Aging and Alcoholism Anthropology. MARC GALANTER (ed.). *Plenum*: 1984. Pp.427. ISBN 0-306-41534-8. \$52.50.

Risk Profiles in Clinical Nephrology. Contributions to Nephrology, Vol.37. G. BUCCIANTI (ed.). *Karger*: 1984. Pp.202. ISBN 3-8055-3739-5. SwFr.90, DM108, \$54.

The Role of Ascorbic Acid in Growth, Differentiation and Metabolism of Plants. Advances in Agricultural Biotechnology. N.J. CHINOY (ed.). *Martinus Nijhoff/Dr. W. Junk*: 1984. Pp.322. ISBN 90-247-2908-4. Dfl.125, \$46.50, £31.75.

Steroids and Endometrial Cancer. Progress in Cancer Research and Therapy, Vol.25. VAERIO MARIA JASONNI *et al.* (eds). *Raven*: 1983. Pp.245. ISBN 0-89004-834-7. Np.

Surgery in America: From the Colonial Era to the Twentieth Century, 2nd edition. A. SCOTT EARLE (ed.). *Praeger*: 1983. Pp.407. ISBN 0-03-061999-8. \$49.95.

Surgery in Gynecological Oncology. Developments in Oncology. A.P.M. HEINTS *et al.* (eds). *Martinus Nijhoff*: 1983. Pp.329. ISBN 0-89838-604-7. Dfl.135, \$52, £34.25.

Textbook of Immunopharmacology. M.M. DALE and J.C. FOREMAN (eds). *Blackwell Scientific*: 1984. Pp.407. ISBN 0-632-00859-8. £18.50.

Therapeutic Control of Inflammatory Diseases. Advances in Inflammation Research, Vol.7. IVAN OTTERNESS *et al.* (eds). *Raven Press*: 1983. ISBN 0-89004-983-1. \$60.

Topics in Molecular Pharmacology, Vol.2. A.S.V. BURGESS and G.C.K. ROBERTS (eds). *Elsevier*: 1983. Pp.210. ISBN 0-444-80495-1. Dfl.180, \$69.25.

New for microscopy

The following pages concentrate on equipment for microscopy. Many of the products will be on view at the Micro '84 exhibition at Bloomsbury Crest Hotel, London, on 9-13 July.

● Imagex is a new data and processing software package which combines the scanning capability of an electron microscope with the computation power and display flexibility of the Kevex Microanalyst 8000. By means of a few simple menus, Imagex controls: acquisition dwell times; data storage size (8 or 16 bits); array sizes and ADC parameters. Images can be created from any digital or analog signal: X-rays; secondary electrons; back-scattered electrons; specimen current; Auger electrons and transmitted electrons. New images can be created from old ones using not only arithmetic functions, but also user-specified logical algorithms.

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● The Philips SEM 505 all-purpose scanning microscope is now available with facilities for the automation of laborious procedures. Machine-intelligent decision making computer interfacing makes possible preprogrammed microanalysis, multi-point metrology, elemental mapping and linewidth measurement (in integrated circuits). The SEM 505 also offers a wide choice of illumination options including tungsten or lanthanum hexaboride emitters and low-voltage capability down to 200 volts EHT, for efficient viewing of delicate or uncoated samples in both biological and materials science applications.

Circle No.101 on Reader Service Card.

● A precise three-dimensional micromanipulator, the World Precision Instruments Model 10000 features a sturdy elevated based for use where additional height is needed. Inverted microscope stages, for example, require a manipulator six inches or more above the table top. The elevated base anchors to the work surface via very strong permanent magnets. The Model 10000 features positional resolution of $0.1\ \mu\text{m}$ and a coarse movement in excess of 1 inch in the x , y and z dimensions. Remote hydraulically coupled micrometers are also available.

Circle No.102 on Reader Service Card.

● The Reichert-McBain System II is a computer-controlled intelligent measuring and positioning system. Designed specially for high-speed precision inspection and measurement of critical dimensions, the system is ideal for linewidth measurements on wafers, hybrids, and masks, and also for measurements of the critical dimensions/alignment of computer read/write heads. MAPS can also be used for end-geometry measurement in optical fibre quality assurance.

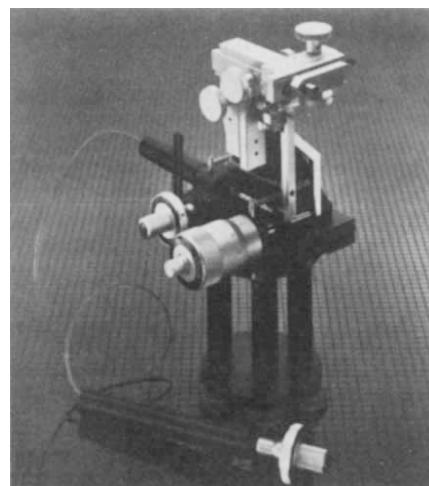
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Kevex Microanalyst 8000.

● The Olympus Vanox-S photographic research microscope incorporates automated control of all adjustments. Intensity, objective selection and Koehler illumination adjustment are automatically set to suit the objective chosen by the operator. Any one of the three cameras, two 35mm and one large format, can be brought into operation and the operator can see exactly what the camera does through the instrument's reflect viewing system.

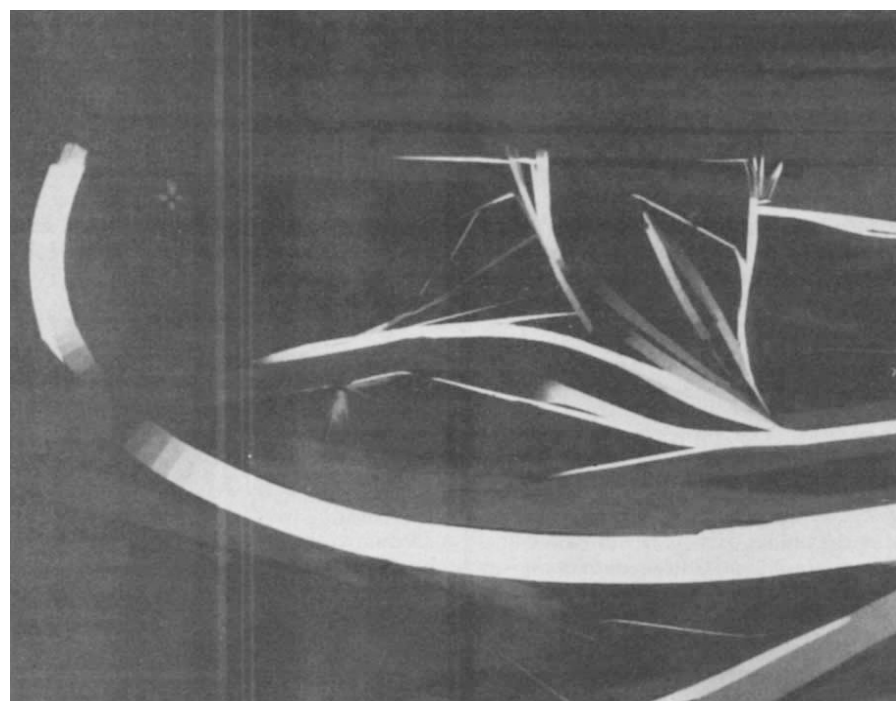
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WPI's new micromanipulator.

● Edax UK has introduced the new Econ IV detecting unit for energy dispersive analysis for all elements in the periodic table from boron (atomic number 5) upwards. Econ IV costs little more than a standard energy dispersive detector, bringing light element analysis capability to a wider spectrum of users. A wide range of electron microscope interfaces is also available.

Circle No.105 on Reader Service Card.



Crystals of niacinamide, photographed at $100\times$ on Polaroid Type 55 film, with 100W halogen illumination through a No.12 Wratten filter using an Olympus BH-2 microscope and Olympus OM-2 camera. This photomicrograph, by David Parker, won a prize in the 1983 Polaroid photomicrography competition. Closing date for the 1984 contest is 5 October. Entry forms are available from Polaroid (UK) Ltd, Ashley Road, Herts AL1 5PR, UK.

● Applications for the Polaron Model 2000 plasma asher/etcher system include mineral particle isolation, surface etching of geological and metallurgical samples, cleansing of delicate items and ultrastructural investigation. The Model 2000, which uses RF excitation of gases to produce a highly reactive plasma at low temperatures, is a simple unit which contains both power supply and reaction chamber. The material to be processed is loaded in to the sample chamber which is then sealed and evacuated, using an external vacuum pump. A reactive gas is then bled in to the reaction chamber through an adjustable leak-valve and excited to the plasma state using up to 100W of RF power at 13.56MHz. Typical gases are oxygen, hydrogen and fluorinated or chlorinated hydrocarbons and typical reaction time 30 min.

Circle No.106 on Reader Service Card.

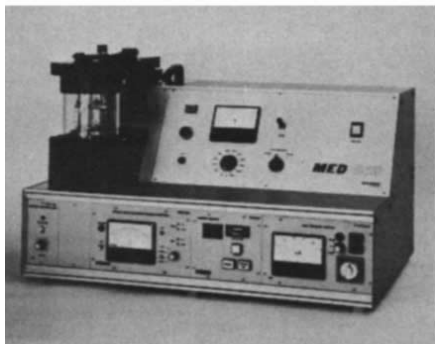
● The latest mini high vacuum deposition system from Balzers, the MED 010 "Turbo", is a bench-top unit suitable for all commonly used TEM and SEM deposition techniques, and features a newly designed safety circuit to increase user protection. Also new from Balzers is the CPD 020 critical point drier. The CPD020 is designed for drying biological specimens which might be damaged by the forces resulting from the surface tension during normal drying. The specimen chamber provides excellent observation having two large sight glasses mounted in both top and side of the vessel. Balzers also market a SCD 030 Sputtering device for the production of conductive films on SEM specimens which avoids undesirable charging of the specimen surface.

Circle No.107 on Reader Service Card.

● Oxford Instruments, specialists in cryogenic and cryomagnetic systems, produces a range of cold stages (8–300K) for transmission and scanning electron microscopes. For cooling down to 80K using liquid nitrogen, the cold stage consists of a 0.65 litre Dewar mounted on the SEM with flexible tubular connections to a special sample mount. Operation is by a continuous flow of liquid under gravity feed to the sample heat exchanger. A cold finger type cryostat is used for temperatures below 10K. The principles of the continuous flow cryostat has also been utilized in cryostages for TEM. Microscope translational movement and eucentric tilting along the holder axis are not impaired and crystallographic work as secondary tilt can be provided. Operation down to 10K is possible with the double tilting stage and to 6K for a single tilt.

Circle No.108 on Reader Service Card.

● The latest model in the CamScan range of scanning electron microscopes, the Series 4, is a modular system which features detectors for secondary, back-scattered and absorbed electrons, optimized geo-



The Polaron E2000 plasma asher (top) and the MED010 high vacuum deposition system from Balzers.

metries for both EDX and WDX spectrometers and an extensive choice of scan, video and image processing functions. Excellent sample handling facilities are provided by the large specimen chamber and the 100 mm fully eucentric stage, multiple accessory ports and a sample viewing window facilitate the integration of accessories for cryogenic stage/specimen transfer airlock for biological materials. Motor drives are available for automated stage control and when operated in conjunction with the EDX/WDX X-ray spectrometer systems give fully computer controlled micro-probe operation of the instrument.

Circle No.109 on Reader Service Card.

● The growing interest in applications of low-temperature SEM for biological and material sciences has led to the development of a reliable and easy to use Cryotransfer system by Hexland. The Hexland CT 1000 Cryotrans system offers cryo observation and analysis by EDS and WDS techniques to be carried out on most SEMs currently available. Adaptation to older SEM's is also possible. The Hexland Cryotrans Preparation system is designed on a modular basis to include an efficient cold stage module, separate anti-contaminator shielding in the SEM, airlock and interface to the SEM chamber. The Cryotrans is simple to operate and requires minimal maintenance. Controlled sublimation or etching is possible whilst viewing the SEM image. The specimen can be coated with sputter or high vacuum evaporation techniques. Monitoring of all cold areas is possible, with accurate temperature control of the SEM cold stage to within $\pm 1^\circ\text{C}$.

Circle No.110 on Reader Service Card.

● The Sorvall MT 6000 is a microprocessor-controlled ultramicrotome, which combines high cutting precision and reproducibility with ease of operation and control. The MT 6000, manufactured by Du Pont, is supplied with a special gear-driven universal specimen holder which allows 360° axial rotation of the specimen as well as movement through a 40° arc in all directions. Other features include a fibre optics lighting system, built-in vibration damping and the Visutrax system, an exclusive feature for tracking the cutting arm movement and for positioning the cutting zone. With the newly introduced cryosectioning system accessory, the Sorvall FS 1000, the MT 6000 ultramicrotome is able to section freshly frozen or freeze-fixed cryoprotected samples consistently. The MT 6000 may be used with the Du Pont diamond knife, available in cutting edge length from 1.0 to 45 mm, with included angles of 45° or 55° .

Circle No.111 on Reader Service Card.

● The new Quantimet 920 image analyser from Cambridge Instruments offers new possibilities in computer-aided autoradiology, oil contamination monitoring and three-dimension image reconstruction applications, as well as the well established applications in mineralogy, metallurgy and biology. Images can be obtained from optical microscopes, photographs, negatives cine and strip films, slides and radiographs, as well as the objects themselves. Direct links are also available to interface the Quantimet 920 with Cambridge Instruments range of scanning electron microscopes. Features of the Quantimet 920 include: dual displays, a flicker-free, high resolution display for imaging and control, a colour display for clear object identification and colour mapping images, and an extensive range of image processing and analysis facilities. Image analysis is achieved quickly and easily by using Cambridge Instruments' QUIPS language for solving research and quality tasks.

Circle No.112 on Reader Service Card.

● The JEM-100SX, the smallest TEM in the JEOL range now features a minimum electron dose sequence as standard, as well as improved objective lens geometry, two new magnification settings in the low magnification range, and three new settings in the high range. Two configurations are available in the JEM-100SX, both with side entry specimen holders. One has $\pm 10^\circ$ specimen tilt, the other, fitted with a eucentric tilting goniometer, gives $\pm 60^\circ$ specimen tilt. The JEM-100SX will resolve 3.4 nm graphite lattice and is designed primarily to meet the biological TEM worker. Now that a minimum electron dose system is fitted, it is particularly useful to examine beam sensitive material by adjustment of the instrument on a specimen region adjacent to that being photographed.

Circle No.113 on Reader Service Card.

● The System 5000 from EG&G Ortec is one of the new generation of energy dispersive spectrometers. It features enhanced computing power allowing almost instantaneous qualitative and quantitative analysis. The analytical software even allows accurate quantitative analysis to be performed on scanning electron microscopes equipped with field emission electron sources. The entire 266 kbytes of RAM in the System 5000 is totally addressable — some older competitive systems only allowed the software to address 64 kbytes of RAM. With the System 5000 it is possible to address up to 1m byte of RAM if desired. All data acquisition functions are under computer control including energy calibration. Six different KLM marker modes are provided as well as a totally automatic qualitative analysis routine.

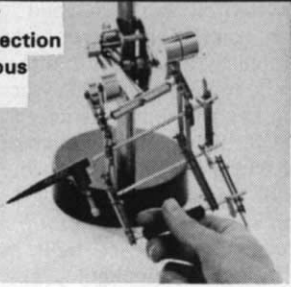
Circle No. 114 on Reader Service Card.

● A new critical point drier, the Emscope CPD 750, is claimed to transform the hitherto tedious process of sample drying at the critical point. The pressure chamber, which is vertical, is illuminated from below. Because the chamber volume has been reduced to minimize CO₂ consumption, unique specimen holders have been developed to maximize the number of samples dried per cycle. A vertical chamber extension can be supplied to accommodate a larger number of samples if required. A thermoelectric device performs a dual function of pre-cooling the chamber to 10°C and heating to the critical temperature. A double-pole switch is fitted to allow selection of thermostatically controlled cooling or heating. In the CPD 750 water circulation is not required, thus simplifying apparatus and removing the reliance upon hot/cold water supplies or a thermocirculator. Agitation of the chamber contents, by a magnetic stirrer, may be used to assist with solvent exchange, thus speeding up the drying cycle.

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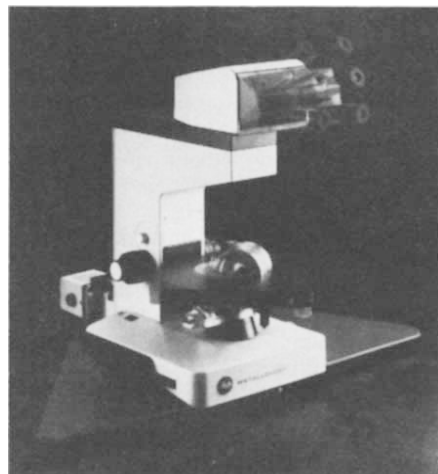
Micro Consultant's Intellect 100 and extras.

● Aimed at the electron microscopist, KIAS (Keypad Image Analysis System) is a particle analysis software system based on Micro Consultants' Intellect 100. KIAS is driven using a keypad in a similar way to the basic Intellect 100 with the RTA (Real Time Analyser) hardware option. The Intellect 100 is designed to accept inputs from normal TV pictures and (with its slow scan option) slow scan devices on equipment, including optical and electron microscopes, thermal imaging systems and X-ray scanners. Also available is a Summagraphics bit-pad touch tablet for manual editing operations. The RTA provides powerful grey level and binary image processing, fast image analysis and noise reduction to clean up the image.

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● The ASM 100 Acoustic Scanning Microscope from VG Semicon is designed to suit the industrial applications of acoustic microscopy. As the technique moves from the university research environment to industrial laboratories the instrumentation must become more sophisticated to allow flexibility and ease of use. Many of the microscope operations are controlled by a powerful 16-bit processor and images are acquired on a 512 × 512 pixel digital frame store. For high frequency microscopy a miniature two-axis lens scanner has been developed which simply fits onto the turret of a conventional optical microscope. There are many applications in the field of non-destructive evaluation that involve the examination of the interior of opaque solid materials, for example, crack detection in welds, delaminations in integrated circuits and void detection in solder bonds. The acoustic scanning microscope obtains images which record the elastic properties of the object and is therefore potentially useful in these areas. The principle of acoustic scanning microscopy is to focus pulsed soundwaves to a diffraction limited spot using a simple spherical lens and then mechanically scan the object or the lens to build up an image. In the ASM 100 this image is acquired on an 8-bit digital frame store allowing subsequent processing, colour encoding or quantitation. The ASM 100 has low frequency imaging capabilities which allow penetration depths of several millimetres for most materials.

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Metallovert, the new inverted microscope from Leitz with variable-angle eyetube and a rotating stage.

● New from Leitz Instruments, the Metallovert provides all the features of a truly inverted metallurgical microscope, while retaining the economic benefits of a conventional upright microscope. The Metallovert has a large field of view index (22.5) and also features a stage that rotates through 115 degrees and can take specimens weighing up to 5 kg.

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● Zeiss Microscope Optics is a 12-page guide to the selection of the microscope objectives, condensers and eyepieces for specific applications, published by Carl Zeiss Inc. In addition to identifying a wide range of objective types, the brochure gives background on diffraction-limited optics design and manufacture. The brochure discusses the reasons for the nearly 150 objectives that are available for Zeiss light microscopes in more than two dozen numerical apertures and nearly four dozen working distances. A listing of fixed, swing-out and turret condensers also includes recommendations for their use in brightfield, darkfield, phase contrast, polarized light and DIC techniques.

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● Tracor Europa has added two new instruments to its range of microanalysis equipment. A low cost fully quantitative EDS system, the TN-5400 series, is a DEC based LSI-11/23 system with 256Kbytes of memory and with options including digital beam scan control and the light element Micro-Z detector. For the user interested in ED XRF, Tracor produces the Spectrace 4020, an easy to use ED XRF analyser. Utilizing an Intel 8086 processor with 256K memory and CP/M86 operating system gives the operator total flexibility for a wide range of commercially available software.

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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● The 'R' range Euromex microscopes are equipped with a modern, heavy stand, designed and manufactured on the basis of the most recent technical developments. The stands are provided with a stove-hardened, high-quality, acid-resistant lacquer enamelling, colour off-white. The parts are anodized or chrome-plated. The table has been sprayed black. The coaxial, low-mounted macro and micrometer knobs are provided with tension adjustment. The micrometer can be used over the full 40 mm range. The multiple-coated prisms in the inclined tubes guarantee optimal light transmission. All objectives and eyepieces meet the DIN specifications. In the MIC 1040 Euromex trinocular microscope RT, the binocular tube is provided with a vertical photo tube. With the phototube switched on, 80% of the light output goes to the camera. The image remains visible through the binocular tube.

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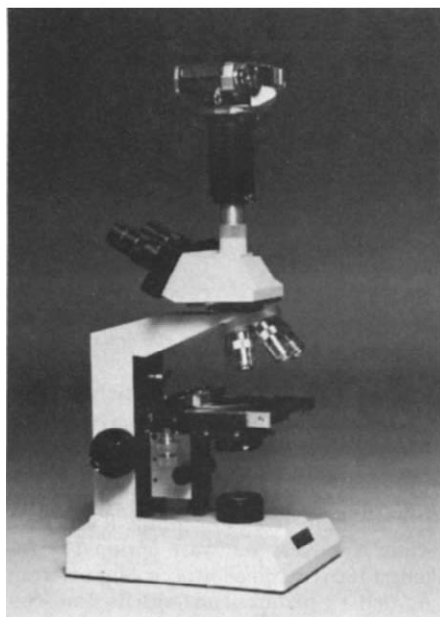
● The Science Photo Library is the world's leading photo agency specialising in all aspects of science, technology and medicine. Its staff are interested in meeting people who are producing high-quality imagery in all fields of microscopy, both in traditional areas and at the frontiers of research. They are also seeking micrographs of all kinds for a book on modern microscopy — *Microcosmos* — to be published in 1986.

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● HistoResin, from LKB Instruments Ltd, is a glycol methacrylate polymer developed to provide an easy-to-use embedding system for high resolution light microscopy. It offers a non-toxic, rapid and economical approach to the production and clear and highly transparent specimen blocks. The HistoResin kit includes all the components required for specimen infiltration and embedding. Basic resin is supplied in a convenient spouted bottle and activator in prepacked envelopes so there is no exposure to caustic chemicals, or spillage or waste. A special mounting medium is included in the HistoResin system to ensure stable, nontacky blocks and secure attachment of virtually any adapter.

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● A macrodual zoom system is available for use with the Reichert-Jung Polyvar when macroscopic examination of large specimens, such as lung or brain, is required. Suitable for use with semi-automatic image analysis systems, the macrodual zoom image can be seen in the binocular tube, the camera system and a TV camera if connected. As an added facility, a drawing surface or an electronic digitizer tablet can be placed under the macrodual zoom. The drawing surface can then be imaged and magnified as required before being superimposed on the microscopic image in the objectives. Using a digitizer tablet and an electronic cursor, the acquisition of structured data is achieved



MIC 1040 Euromex trinocular microscope RT with camera.

by scanning the microscopic image which is simultaneously visible in the viewing tube. It is also possible to carry out accurate measurements with the macrodual zoom, and the macrodual-zoom can also be used to identify structures for later discussion using a digit projector.

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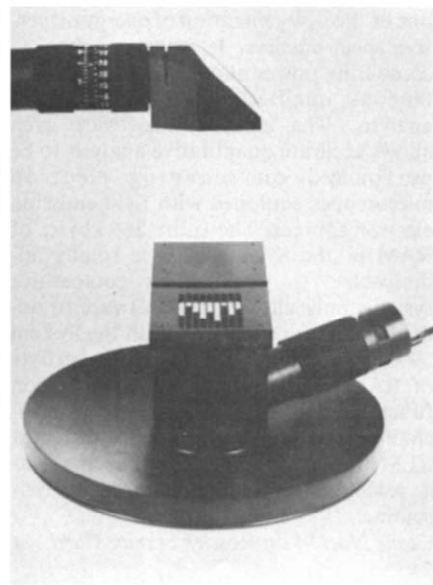
● A new, free 12 page product brochure describing the latest developments in biologicals, microscopy, monomers/polymers, and life sciences is now available from Polysciences Inc. Featured products include: fluorescent probes and fluorogenic substrates; synthetic agar; fungi-fluor kit; new reference books; new monomers including phenyl vinyl sulphone; and acrylamide-bis premixes.

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● A range of reusable transit containers for delicate instrumentation is now available from Skypak Containers Ltd. They are ideal for field analysers, demonstration instruments and equipment regularly transported between laboratories. Skypak manufacture each container for the individual application. The size, the internal furnishings, the external fittings, and even the colour can be chosen to match the user's specifications. Melamine or aluminium-faced plywood panels with PVC surrounds provide weatherproof, shock resistant protection. Shaped internal furnishings of non-hydroscopic polyethylene foam provide attractive, thermally stable cushioning, resistant to oils, petrols, acids and alkalis. All external fittings and handles lay flush with the sides of the box.

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● The easiest and most reliable means of ultracleaning or sterilising small volumes of liquids is to use a disposable syringe filter holder. But the requirements for different operations dictate different pore size



Macroductal zoom with digit projector from Reichert-Jung.

filters. To provide users with this choice, the Minisart range available from Semat (UK) has been extended to include 0.2, 0.45 and 0.8 μm filter units. As an additional safety feature each pore size has been produced in a different colour (blue for 0.2, yellow for 0.45 and green for 0.8 μm avoiding user mistake in picking up the wrong filter unit. The advantages of the Minisart range include: high resistance to pressure; minimal dead volume; superior flow rates; minimal adsorption; individually packaged, sterile, pyrogen — free units; medically safe materials (proven non-toxicity); reliability; inlet Luer lock; back pressure support; and reduced filtration costs.

Circle No. 127 on Reader Service Card.

● Polaron Equipment Ltd have introduced a new range of immunolabelling reagents for light and electron microscopy. The premier range is comprehensive and includes unconjugated colloidal gold and colloidal gold coated with protein A, avidin, biotin, antibodies and lectins. A typical example of these techniques is illustrated by avidin and biotin, two new reagents used in immunocytochemistry. Avidin has a very high affinity for biotin; one molecule can bind four biotin molecules. Biotin will also bind to the Fc fragment of immunoglobulins. A variety of combinations of avidin, biotin and antibody can therefore be built up. When labelled with colloidal gold, these binding sites are clearly demonstrated, both in scanning electron microscopy and light microscopy, due to colloidal gold's good emission of secondary electrons and strong red colour in white light. Each reagent is available in particles, 10–15 nm and 20–25 nm, enabling comparative localization studies to be performed on a single specimen.

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